

Supporting Information

Material and Methods

From capture to maintenance in experimental tanks

Fishing procedures were optimized following Peleteiro et al. (2004) and preliminary tests. Briefly, sardines were concentrated by tightening the net, coaxed gently into buckets so that they were always immersed in water then transferred immediately into 1 m³ tanks of oxygenated seawater on board. After boat and lorry transport to the IFREMER experimental station at Palavas-les-Flots (Hérault, France), fish were held in outdoor tanks (4.5m³) supplied with a flow of aerated local seawater at prevailing temperature and photoperiod. The whole operation, from capture to outdoor tanks, took less than 4 hours. The first 2-3 days following capture constituted the most critical period of acclimation, but this varied quite a lot from one fishing event to another, probably due to the sea temperature when the fishing was carried out (the higher the sea temperature, the higher the mortality in the few days after fishing). During the first 5 days, daily prophylactic baths of oxytetracycline (100 ppm) were administered to prevent bacterial infections from fishing injury and scale loss. Over the first week, sardines were fed both *Artemia nauplii* and aquaculture pellets (mix of pellet sizes: 0.1mm, 0.3mm and 0.8mm), with increasing proportions of pellets and decreasing proportions of *Artemia* throughout the week, concluding with meals exclusively of pellets. Pellets were distributed by automatic feeders throughout the day whereas *Artemia* meals were provided once, in the morning. To maximize survival, food rates were high (between 2% and 6% of biomass), such that body condition increased from a mean (\pm SD) of 1.0 (\pm 0.1) at fishing to 1.2 (\pm 0.1) at the start of experiments. Natural swimming and schooling behavior occurred within a few days of capture. After 2 to 3 weeks acclimation (depending on fishing dates) and upon confirmation of the absence of NODA virus, sardines were moved into indoor tanks for experiments.

Feeding conditions

As in most experimental studies, we tried to be as close as possible to the natural environment (i.e. tanks were supplied with water pumped from the sea, followed natural photo- and thermo-period) to have natural behavior of sardines. The number and density of fish per tank allowed for the formation of schools (Fig. S2 and Movie S1). To control accurately the quality, quantity and size of the food along the 7-month of the experiment, the use of living prey would not have been appropriate and we therefore chose to feed sardines with

35 standardized aquaculture pellets having the same quality in terms of lipid and protein
36 contents. Both pellet sizes shared similar lipid class contents except for the phospholipid class
37 (see Table S1). Moreover, the two pellet sizes corresponded to main prey sizes found in the
38 wild during those two periods (Le Bourg et al., 2015). Although we are aware of the
39 limitation of inert food versus live prey, normal feeding behavior on aquaculture pellets was
40 ensured before the start of the study (Movie S1). Finally, based on the mean age at the
41 beginning of the experimentation (i.e. 1.2 years) and on the von Bertalanffy curves adjusted
42 by Van Beveren et al. (2014), monthly growth rates in captivity were similar to the ones in the
43 wild (around 1.5 mm month⁻¹ and between 0.5 and 2.5 mm month⁻¹, in Van Beveren et al.
44 (2014) and this study, respectively).

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47 **Estimation of food loss**

48 To quantify loss of non-ingested food, we performed an additional 2-week experiment in a
49 single tank, with a similar density of sardines to the previous experiment, where we estimated
50 the quantity of food that (i) deposited on the bottom of the tank and (ii) left the tank through a
51 bottom grid. To do so, 5 glass Petri dishes were placed on the bottom of the tank before each
52 meal (total tank area = 76.6 x 10⁻² m², bottom grid area = 15.6 x 10⁻² m², dish area = 1.45 x
53 10⁻² m²) and a 70-µm-mesh sieve used to filter outflowing water (Fig. S3). The experiment
54 comprised 16 meals (4 meals per day) distributed over 10 days. Fish were fed 1.2mm pellet in
55 large quantity on days 1 and 3, fasted on days 2 and 4. Fish were acclimated to 0.1mm pellet
56 on days 5 and 6, then days 7 and 8 repeated days 1 and 2 but using the smaller pellet in large
57 quantity, with days 9 and 10 repeating days 7 and 8. Petri dishes and sieve were removed for
58 analysis 90 minutes after each meal.

59 Samples from Petri dishes and the sieve were then filtered through a 0.7-µm dry filter (dried
60 beforehand at 60°C in the autoclave for 24 hours and weighed to the nearest 0.0001g), then
61 filters were rinsed with distilled water to remove salt. After manually removing faeces and
62 scales, filters were dried at 60°C in the autoclave for 24 hours and then weighed, so that mass
63 of matter could be estimated as the difference in filter dry weights. The total mass of collected
64 matter was the sum of matter collected by the sieve and matter collected by the 5 dishes
65 weighted by the ratio of tank surface to dish surface (without the bottom grid area):

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$$67 \text{ collected matter} = \sum \text{collected matter}_{\text{dish}} \times \frac{S_{\text{tank}} - S_{\text{bottom grid}}}{\sum S_{\text{dish}}} + \text{collected matter}_{\text{sieve}} \quad [1]$$

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70 Finally, the non-ingested food was estimated as the quantity of collected matter during a meal
71 corrected by the mean quantity of collected matter while fish were fasting and expressed as a
72 fraction of the meal size:

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$$74 \text{ non-ingested food} = \frac{\text{collected matter}_{\text{meal}} - \text{collected matter}_{\text{fasting}}}{\text{meal size}} \times 100 \quad [2]$$

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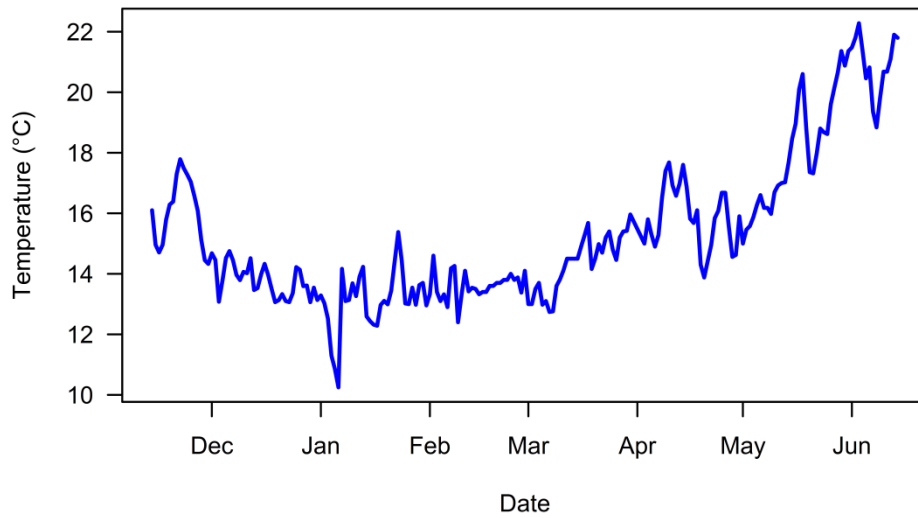
82 **Results**

83 **Body condition and total length over time: mixed-effect models**

84 The impact of the different treatments on body condition and total length were tested using
85 linear mixed-effect models with both random slopes and intercepts for both individuals and
86 tanks (to study tank effect among treatments). The model selection process was following
87 recommendations provided by Burnham and Anderson (2002) and Zuur et al. (2009). The
88 selected model is presented here using REML estimation. Selected models and violin plots of
89 body condition and total length distributions are presented in Fig. S4. For the body condition
90 index, the validation graphs are presented in Fig. S5. Homogeneity, normality and
91 independence were checked through plots of Fig. S5 and only 3 individuals are considered as
92 outliers (i.e. <1% of all individuals) (Fig. S5B). The results of the selected model are
93 presented in Table S2. Simultaneously, the same process was used for the total length
94 parameter. The validation graphs are presented in Fig. S6. Homogeneity, normality and
95 independence were checked through plots of Fig. S6. The results of this selected model are
96 presented in Table S3.

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100 **Fig. S1: Evolution of the water temperature in tanks throughout the 7-month**
101 **experiment**

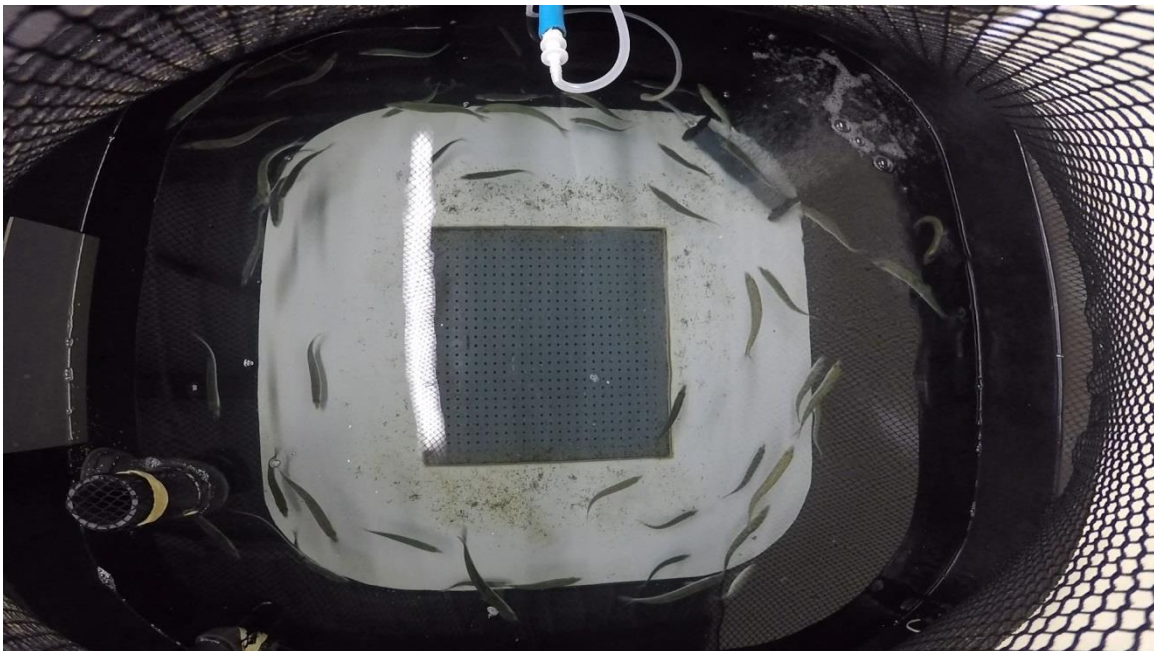
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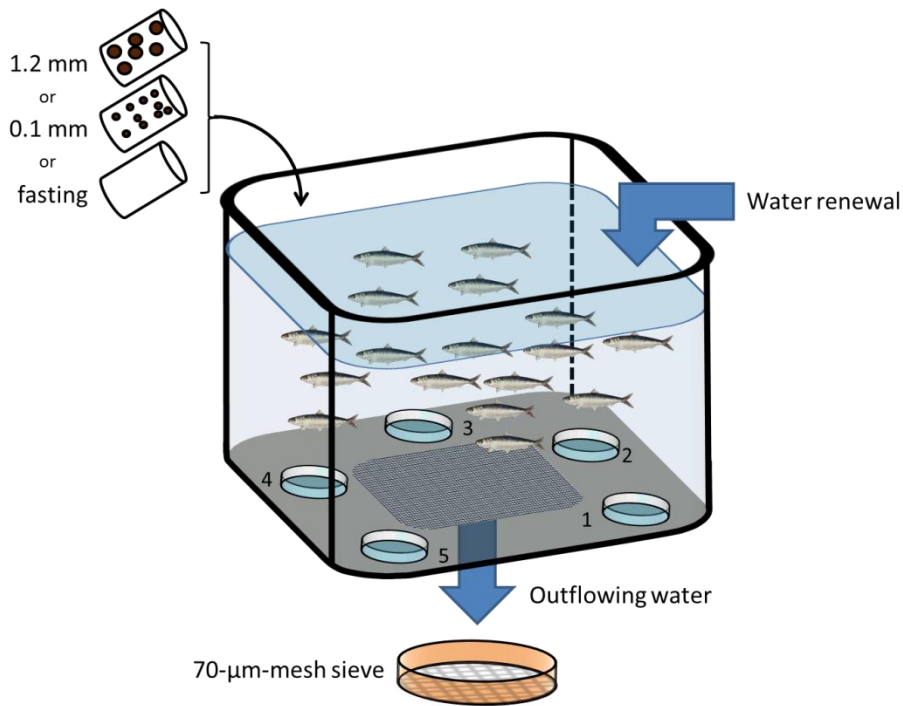
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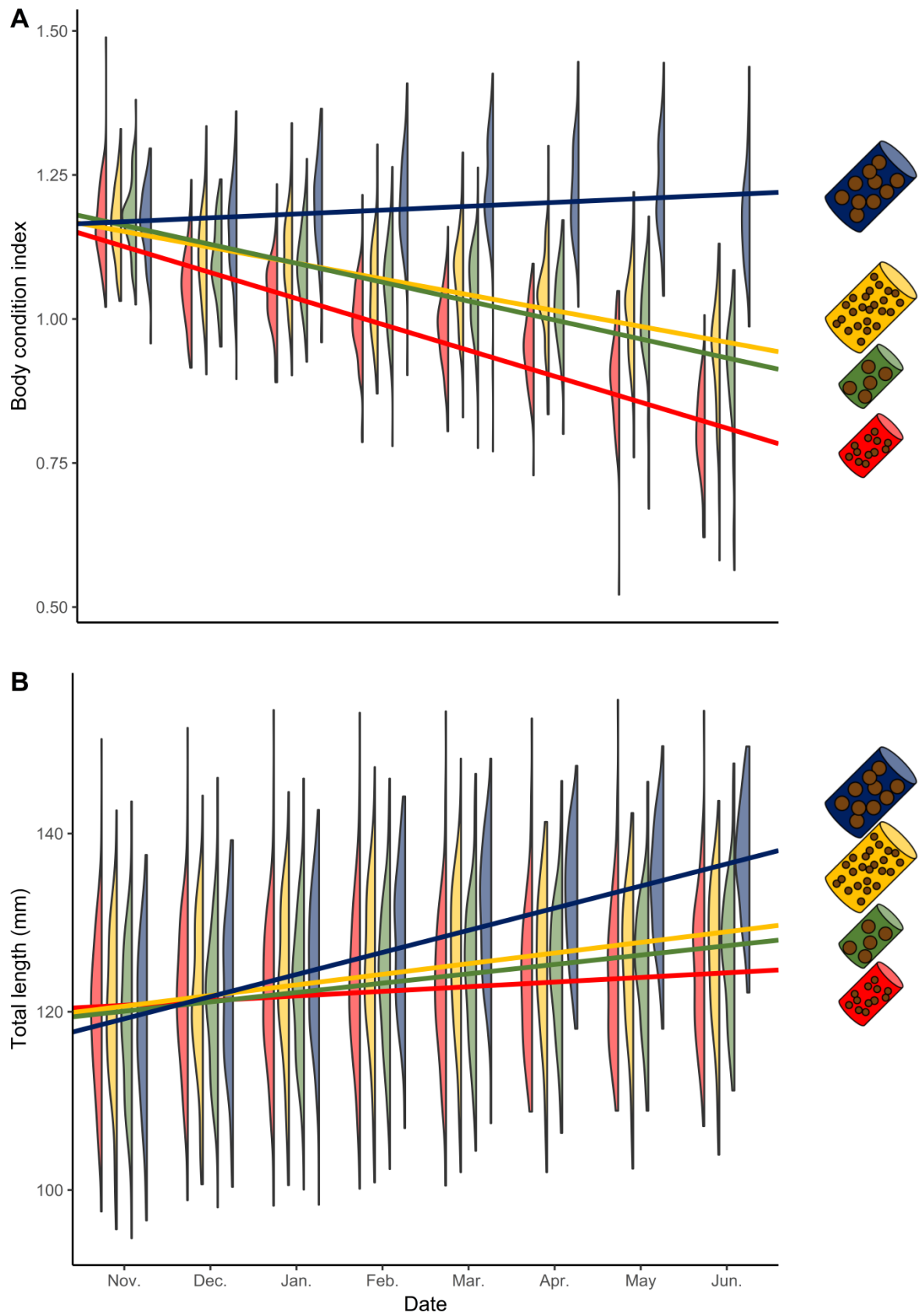
110 **Fig. S2: Picture of the swimming and schooling behavior of sardines in the 300 L**
111 **experimental tank**

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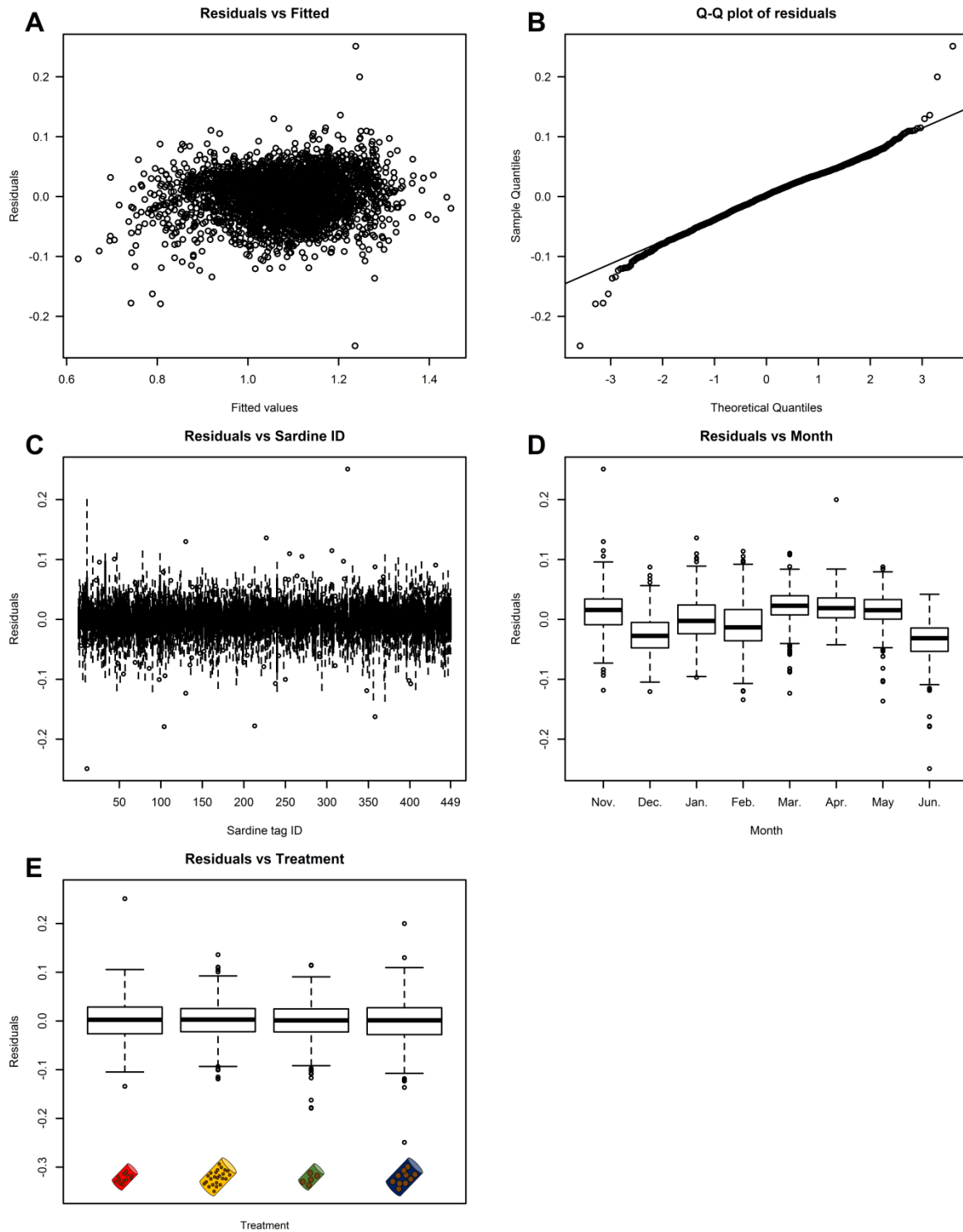
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Fig. S3: Experimental design to estimate the non-ingested food



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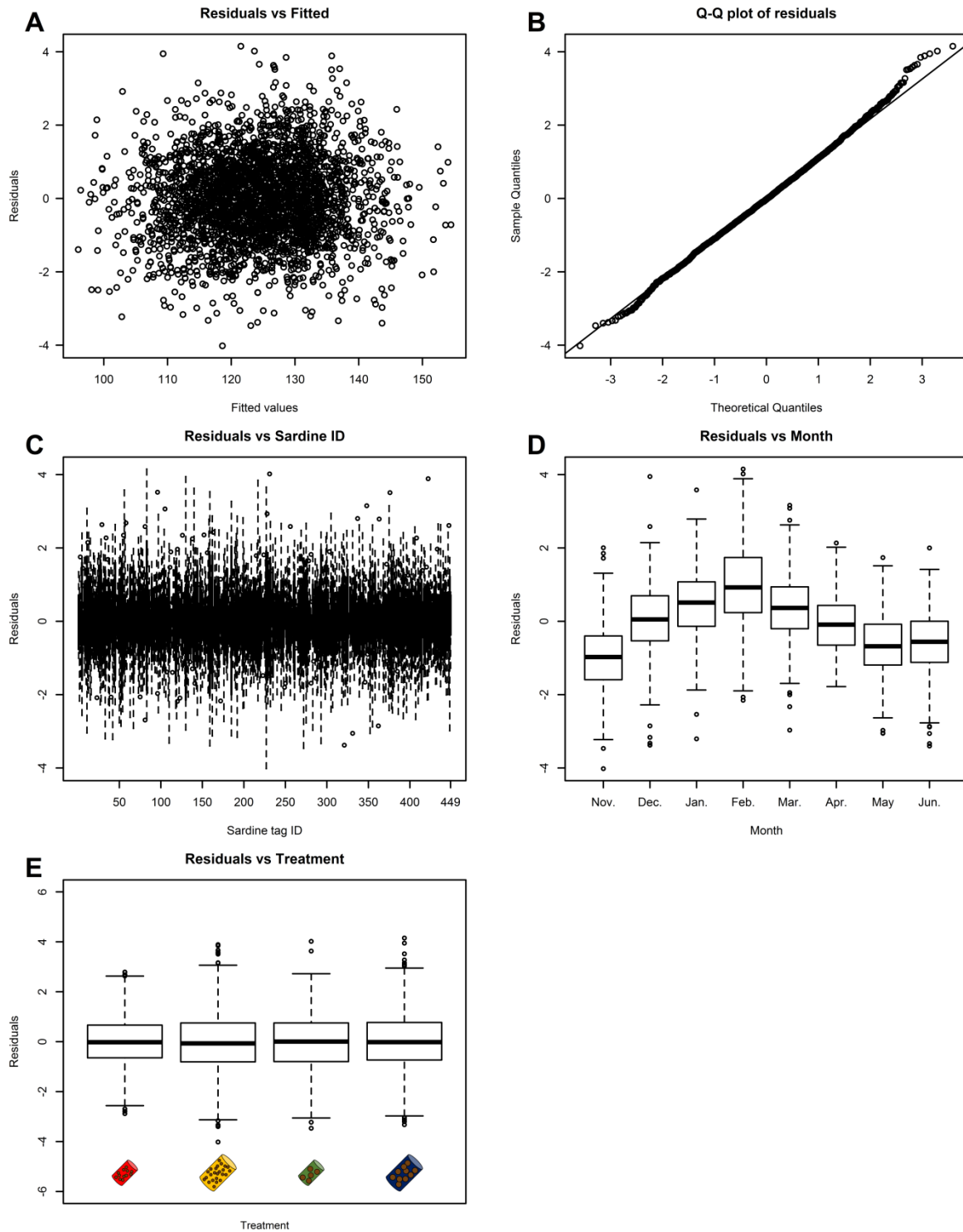
Fig. S4: Violin plots of body condition index (A) and total length (B) distributions over time. Selected linear mixed-effect models were added (lines).



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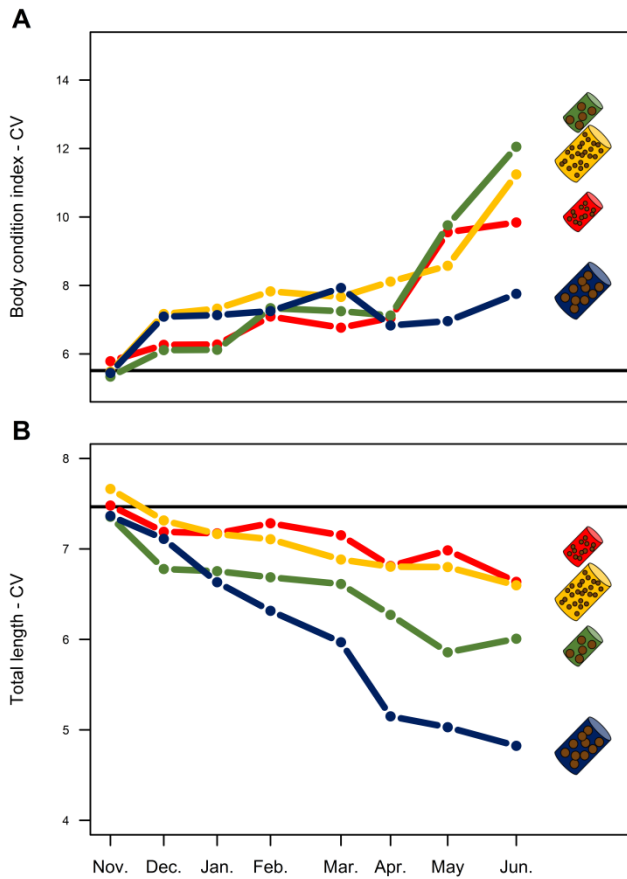
124 **Fig. S5: Validation graphs of the selected body condition model. A: Fitted values versus**
 125 **residuals. B: Q-Q plot of the residuals. C: Residuals versus sardine ID. D: Residuals**
 126 **versus time (month). E: Residuals versus treatments**

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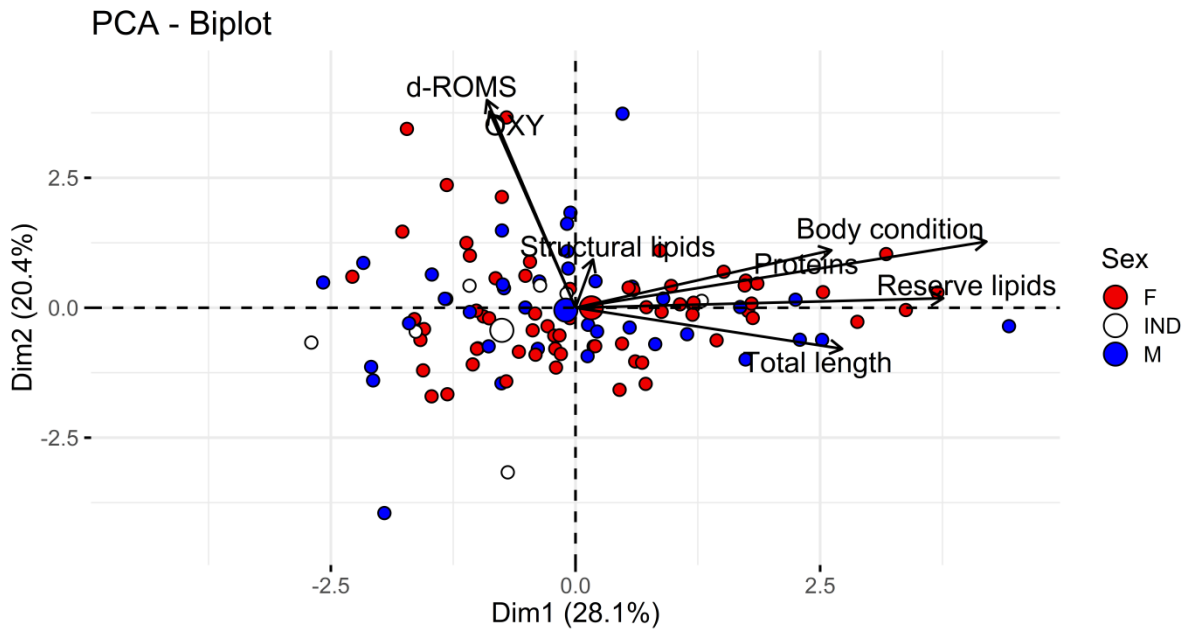
132 **Fig. S6: Validation graphs of the selected total length model. A: Fitted values versus**
 133 **residuals. B: Q-Q plot of the residuals. C: Residuals versus sardine ID. D: Residuals**
 134 **versus time (month). E: Residuals versus treatments**
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139 **Fig. S7: Time series of the CV of the body condition (A) and total length (B) of all**
 140 **sardines in each feeding treatment: red: pellet size of 0.1mm and pellet quantity of**
 141 **0.3%; green: 0.1mm and 0.6%; yellow: 1.2mm and 0.1% and blue: 1.2mm and 0.6%.**
 142 **Dark line is the mean body condition and total length at the beginning of the**
 143 **experiments.**

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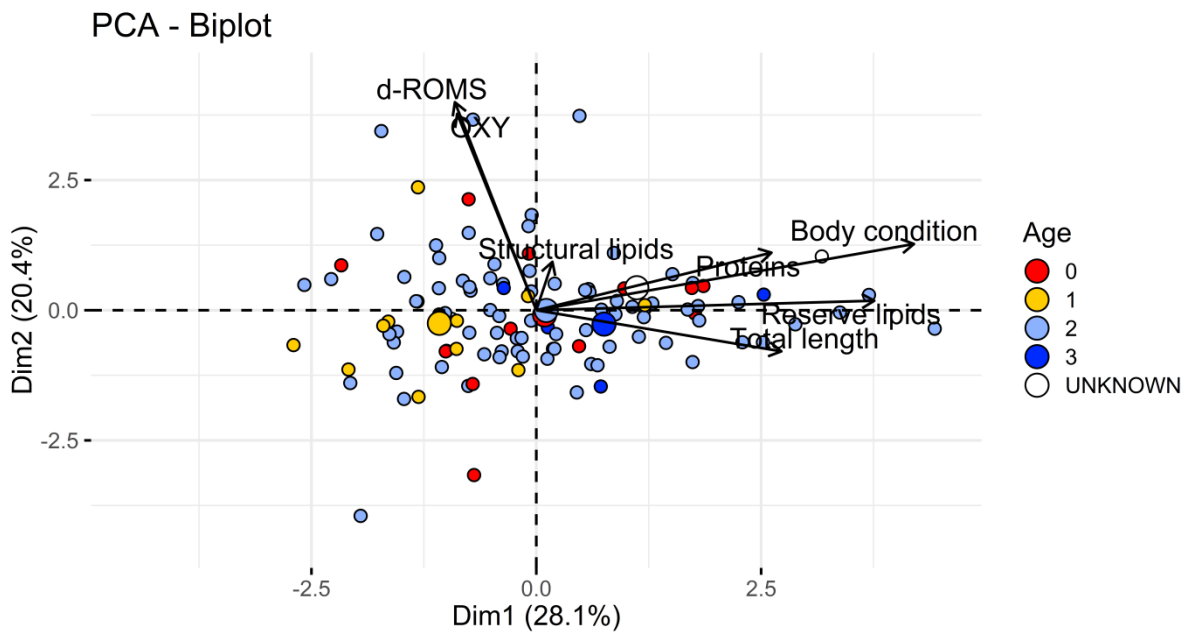


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147 **Fig. S8: Biplot of the PCA built using body condition, total length, reserve and**
 148 **structural lipids and proteins contents, d-ROMS and OXY as explanatory variables,**
 149 **with grouping by sex. The large circles represent the barycenter of the individuals for a**
 150 **given sex.**

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154 **Fig. S9: Biplot of the PCA built using body condition, total length, reserve and**
 155 **structural lipids and proteins contents, d-ROMS and OXY as explanatory variables,**
 156 **with grouping by age. The large circles represent the barycenter of the individuals for a**
 157 **given age.**

158 **Table S1. Mean \pm sd of lipid class contents ($\mu\text{g}/\text{mg}$) of 0.1 and 1.2 mm pellets used in this**
159 **study: triacylglycerols (TAG), free fatty acids (FFA), alcohols (ALC), sterols (ST),**
160 **acetone-mobile polar lipids (AMPL), diacylglycerols (DAG) and phospholipids (PL).**
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Pellet size (mm)	TAG	FFA	ALC	ST	AMPL	DAG	PL
0.1	67.1 \pm 13.5	4.6 \pm 4.3	3.5 \pm 2.7	7.0 \pm 5.0	12.0 \pm 6.6	2.7 \pm 2.5	55.7 \pm 13.7
1.2	69.0 \pm 24.6	3.6 \pm 4.1	2.9 \pm 2.6	5.7 \pm 4.9	10.4 \pm 11.3	2.1 \pm 2.4	24.6 \pm 14.3

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163 **Table S2. Results of the selected mixed effect model of body condition over time.**
 164 **Estimations of the predictors of all other fixed effects were based on the estimations of**
 165 **treatment 1. For instance, the intercept of treatment 4 (BLUE) was +1.18 (i.e. 1.14+0.04)**
 166 **and the slope was +0.01 (i.e. -0.04+0.05).**
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Random effects:

	<i>Standard deviation</i>	<i>Correlation</i>
(Intercept)	0.06	(Intercept)
Date	0.01	-0.17

Fixed effects:

<i>Predictors</i>	<i>Estimates</i>	<i>95% CI</i>	<i>df</i>	<i>p-value</i>
(Intercept)	1.14	1.12 – 1.15	2565	<0.001
Date	-0.04	-0.05 – -0.04	2565	<0.001
Treatment 2 (YELLOW)	0.02	-0.00 – 0.04	445	0.050
Treatment 3 (GREEN)	0.02	0.01 – 0.04	445	0.012
Treatment 4 (BLUE)	0.04	0.02 – 0.05	445	<0.001
Date:Treatment 2 (YELLOW)	0.02	0.01 – 0.02	2565	<0.001
Date:Treatment 3 (GREEN)	0.01	0.01 – 0.02	2565	<0.001
Date:Treatment 4 (BLUE)	0.05	0.05 – 0.06	2565	<0.001

Observations	3018
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169 **Table S3. Results of the selected mixed effect model of total length over time.**
 170 **Estimations of the predictors of all other fixed effects were based on the estimations of**
 171 **treatment 1. For instance, the intercept of treatment 4 (BLUE) was 119.87 mm (i.e.**
 172 **120.59-0.72) and the slope was +2.48 (i.e. 0.52+1.96).**
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Random effects:

	<i>Standard deviation</i>	<i>Correlation</i>
(Intercept)	8.84	(Intercept)
Date	0.61	-0.35

Fixed effects:

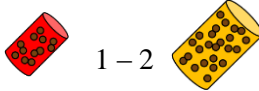
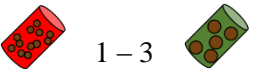
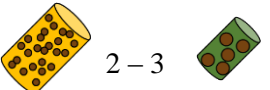
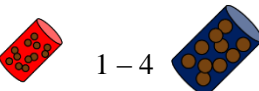

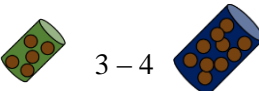
<i>Predictors</i>	<i>Estimates</i>	<i>95% CI</i>	<i>df</i>	<i>p-value</i>
(Intercept)	120.59	118.95 – 122.24	2565	<0.001
Date	0.52	0.39 – 0.65	2565	<0.001
Treatment 2 (YELLOW)	-0.06	-2.39 – 2.27	445	0.959
Treatment 3 (GREEN)	-0.44	-2.77 – 1.90	445	0.713
Treatment 4 (BLUE)	-0.72	-3.05 – 1.61	445	0.543
Date:Treatment 2 (YELLOW)	0.67	0.49 – 0.85	2565	<0.001
Date:Treatment 3 (GREEN)	0.53	0.35 – 0.71	2565	<0.001
Date:Treatment 4 (BLUE)	1.96	1.78 – 2.14	2565	<0.001

Observations	3018
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174 **Table S4. p-values of multiple pairwise comparisons between the four treatments for**
 175 **reserve and structural lipids, proteins, d-ROMS and OXY in March and June.**

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Treatments	Reserve lipids		Structural lipids		Proteins		d-ROMS		OXY	
	March	June	March	June	March	June	March	June	March	June
 1 – 2	0.462	0.741	0.702	0.979	0.684	0.481	1.000	0.150	1.000	0.219
 1 – 3	0.471	0.545	0.068	0.374	0.782	0.768	1.000	0.137	1.000	0.550
 2 – 3	0.808	0.964	0.513	0.710	0.945	0.545	1.000	0.773	1.000	0.837
 1 – 4	<0.001	<0.001	0.051	0.642	0.129	0.053	1.000	0.001	1.000	0.061
 2 – 4	<0.001	<0.001	0.567	0.825	0.290	0.513	1.000	0.093	0.867	0.546
 3 – 4	<0.001	<0.001	0.850	0.085	0.946	0.067	0.868	0.180	1.000	0.596

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182 **Movies**

183 Movie S1: Feeding behavior of sardines fed with aquaculture pellets after acclimation

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189 **References**

- 190
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