

# The use of stomach fullness and colour indices to assess *Sardina pilchardus* feeding

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Scales for stomach fullness and colour were developed and calibrated in order to provide an easy and reliable way to determine feeding intensity and food quality in sardines. The categories of the fullness scale reproduce the amount of food intake as indicated by the weight of the stomachs. The levels of the colour scale reflect the type of plankton eaten as shown by concentration of *a*-type phaeopigments and prey analysis of the stomach contents. Individuals of a wide length range were used in this study, leading us to suggest that these indices can be applied to the entire juvenile and adult sardine population. The use of the colour and fullness scales provides a rapid and efficient means of characterizing sardine feeding. Based on the colour and fullness categories of the stomachs the majority of stomachs were almost empty or at most half-full and the diet was composed of different proportions of phyto- and zooplankton items. As indicated by the prey analyses of the contents the most important constituent of the diet, in volume, were zooplankton prey.

## INTRODUCTION

Sardine (*Sardina pilchardus* Walbaum) is one of the most important fisheries associated with the north-east Atlantic upwelling system, which led to considerable interest in the study of this species particularly the reason for the variability of their abundance. Since food is a principal factor regulating growth, abundance and migration, information on feeding habits and ecology are critical for understanding the dynamics of this resource.

In upwelling areas the pelagic food chain is believed to be relatively simple and short with large zooplankton or fish, mostly clupeids, feeding directly on the large primary producers (Ryther, 1969; Blaxter & Hunter, 1982). However, typical clupeids of upwelling regions like sardines and anchovies are not exclusively herbivorous. They have flexible and opportunistic feeding behaviour that enables them to forage efficiently over a wide range of particle sizes (see James, 1988 for a review). Both are microphages capable of particulate and filter-feeding, although sardines are morphologically better suited to capture and process small particles than anchovies (Cushing, 1978; Blaxter & Hunter, 1982). Following the example of adult *Sardinops sagax* in the southern Benguela upwelling system (van der Lingen, 1994), adult *Sardina pilchardus* in the north-east Atlantic upwelling region probably filter-feeds on phytoplankton, microzooplankton and at high concentrations of large particles while low concentrations of large prey items elicit particulate feeding.

Off the Iberian Peninsula adult *S. pilchardus* stomach contents reflect the dominant plankton groups in the water column (Varela et al., 1988) suggesting that this species obtains the majority of its food through non selective feeding (Varela et al., 1990). More recently Bode et al. (2003, 2004), working with natural abundance of

stable carbon and nitrogen isotopes, both in natural plankton and in sardines, inferred that they must be mainly zoophagous to achieve the isotopic abundance found in the muscle.

Ecological studies involving the relationship of sardine distribution to food availability over extensive areas require analyses of very large numbers of individuals. Stomach content analysis is, however, hard work, it is time consuming and impossible to perform in a large-scale study. It is essential that the methodology used should be easy to carry out during regular biological sampling, either on cruises or at fishing ports, and at the same time be a reliable estimator of feeding intensity and quality.

With these requirements in mind, indices for sardine stomach fullness and colour were developed. The rationale was that fullness reflects the quantity of food while the colour of the stomach indicates the kind of plankton sardines are eating, i.e. a stomach full of zooplankton would be orange and of phytoplankton would be green. Stomach fullness indices are widely used in fishery research not only on pelagic (e.g. Laptikhovskiy, 2002) but also in demersal species (e.g. Velasco & Olaso, 1998), although no reference was found for the calibration of these scales or to the use of stomach scales in sardines. No reference was found for the use of colour scales. The use of indices of fullness and colour instead of stomach content analyses (weighing and identification of items) permits easy processing of a large number of fish and provides an efficient means of characterizing feeding. Continued monitoring of stomachs over time may lead to identification of seasonal and geographical variability of food quality and abundance which in turn may be linked to differences in fecundity, quality of eggs, behaviour and ultimately to variability in the spawning stock biomass.

## MATERIALS AND METHODS

Indices for colour and fullness were created for sardine stomachs.\* The colour scale had four categories: 1, beige; 2, orange; 3, brown; 4, green. The fullness scale was divided in five levels: 0, empty; 1, almost empty; 2, half full; 3, full; 4, bursting. Fullness and colour of stomach contents were independently assessed with reference to the proposed standard by people who routinely perform sardine biological sampling in order to establish what colour and fullness levels represent in terms of phytoplankton content and weight.

Sardines were collected off the coast of Portugal (42°N to 37°N) during two research cruises (November 2002 and February 2003) and from fortnightly fish landings (January to May 2003) at two commercial ports at northern and southern Portugal. Biological data such as length, gutted weight, sex and maturity stage and indices of fat content were registered for each sardine. A total of 970 stomachs were assessed with respect to colour and fullness indices in sardines ranging from 8.5 to 23.2 cm total length which corresponded to ages ranging from 0 to 6+ years. The colour and fullness charts under evaluation were used to classify individual stomachs, after which the stomach was removed for total, wall and content weights estimation with a 0.1 mg precision. Stomach contents were individually deep-frozen for subsequent phytoplankton pigments and prey analysis.

Calibration of the fullness scale was based on the weight of stomach contents. Due to differences in size of stomachs, related to the length and age of each fish, results are expressed in terms of stomach content weight divided by sardine gutted weight.

For the calibration of colour indices, 222 stomach contents were analysed for *a*-type phaeopigments by fluorometry and 41 for microscopic inspection of prey items. Since fluorometry is very sensitive to colour interference and sardine stomach contents were coloured, only a small portion of the content was used for the analysis to prevent erroneous fluorometric readings. The total stomach content was first vigorously blended in a tube, after which a small sub-sample (<1% of the stomach content weight) was taken and weighed to permit calculation of the relative weight. Chlorophyll-*a* (Chl-*a*) and its derivatives (i.e. *a*-type phaeopigments) were extracted with 10 ml 90% acetone solution during 24 h in a freezer and measured with a Perkin-Elmer 204 fluorometer. This instrument was calibrated for Chl-*a* and its acidification product, phaeophytin-*a*. Since stomach contents might contain organic products whose fluorescence spectra could overlap with that of Chl-*a*, a set of readings was first performed using the contents of different colour category stomachs as a matrix to which known concentrations of pure standard Chl-*a* was added. The return readings before and after acidification were proportional to the amount of added Chl-*a* (differences <10%), indicating no significant interference of other products with the Chl-*a* readings.

Chlorophyll and phaeopigments by unit of stomach weight were determined using the following relations adapted from Yentsch & Menzel (1963):

$$\text{Chl-}a = F_d \cdot [\tau/(\tau - 1)] \cdot (F_b - F_a) \cdot F_o/F_i \cdot 10/SW \quad (1)$$

and

$$\text{Phaeo-}a = \{F_d \cdot [\tau/(\tau - 1)] \cdot [\tau \cdot (F_b - F_a)] \cdot F_o/F_i \cdot 10/SW \quad (2)$$

where Chl-*a* and Phaeo-*a* are respectively the chlorophyll-*a* and phaeopigment-*a* concentrations in the stomach contents ( $\mu\text{g g}^{-1}$ ),  $F_d$  is the door factor of the fluorometer ( $\mu\text{g l}^{-1}$ ),  $\tau$  is the acidification ratio,  $F_b$  and  $F_a$  are respectively the fluorescence readings before and after acidification,  $F_o$  and  $F_i$  are respectively the fluorescence of a standard solution on the calibration date and on the actual date, SW is the weight of the stomach content sub-sample (mg) and 10 is a constant for unit conversion.

Microscopic analysis of food items was performed in sets of 5–10 stomach contents for each colour index. Stomach contents belonging to the same colour category were combined and the total weight determined. A small aliquot was then used to count and identify the prey items. The aliquot was first weighed and then diluted in a known volume of water and filtered through a 200- $\mu\text{m}$  sieve to facilitate the identification of the food items. The two size fractions were analysed separately, the fraction <200  $\mu\text{m}$  was identified using an inverted microscope (400 $\times$ ) and the fraction >200  $\mu\text{m}$  with a stereomicroscope (80 $\times$ ) and the number of organisms was determined and expressed as individuals per stomach content gram. Video pictures of the phytoplankton organisms were taken using the software Zeiss KS100 version 3.0 on the inversion microscope. Cells were measured (major and minor axis) and these measurements were converted into volumetric estimates, assuming a spherical or ellipsoidal shape. In the case of zooplankton, whose bodies were in general damaged, the mean length was determined from the literature. Individual body volumes were estimated from the equation given by Valdés et al. (1990) for the neritic zooplankton off north-western Spain and assuming equality between weight measured in  $\mu\text{g}$  and volume measured in  $0.001 \text{ mm}^3$ . In the case of copepods an exponential equation relating copepod mean length to mean volume was determined from data given by Halliday (2001) using the morphometric method and applied to calculate the total volume of each genus or species. The equation was:

$$y = 0.0446 \cdot x^{2.9456} \quad (r^2 = 0.9815; N = 18) \quad (3)$$

where  $y$  is the calanoids' mean volume ( $\text{mm}^3$ ) and  $x$  their mean length in mm.

In order to test the null hypothesis that the fullness index in which each stomach was classified was independent from the colour level, the data were arranged in a contingency table and a chi-square test for independence was selected.

Single-factor analysis of variance (ANOVA) was used to test stomach mass and Phaeo-*a* concentration differences between the indices of the fullness and colour scales respectively. Since inspection of data indicated that the standard deviation of the factors varied proportionally to the mean, statistical analyses were performed on

\*, Photographs of stomachs at each scale level can be requested by e-mail to the corresponding author.

log-transformed values, to stabilize the variance. Multiple comparisons of means between pairs of categories were tested using the Tukey test for unequal sample sizes (Zar, 1999) at confidence level of  $P < 0.05$ .

For comparison of percentages of Chl-*a* degradation, the Freeman & Tukey (1950) transformation was used, since it uses proportions rather than percentages, which is preferable for small and large proportions (Zar, 1999). The data was grouped a priori into nine different concentration classes ( $200 \mu\text{g g}^{-1}$  each) and ANOVA and Tukey tests were also applied. Therefore Chl-*a* degradation corresponded to the Freeman & Tukey (1950) transformed values. This transformation produces values close to double the ratio between Phaeo-*a* and total pigment-*a*, i.e. Phaeo-*a* + Chl-*a*.

## RESULTS

### *Relation of stomach content to stomach fullness and phytoplankton concentration*

For sardines, the relation of stomach total weight to the weight of the contents was:

$$CW = 0.927 \cdot SW - 0.2799 (r^2 = 0.99, \{N = 970, \{P \ll 0.001\}) \quad (4)$$

where *CW* is the wet weight of the stomach contents and *SW* is the stomach total wet weight, both values expressed in grams. These stomachs corresponded to the entire sardine population analysed which ranged from 8.7 to 23.2 cm total length. The good relation means that observations of stomachs are not influenced by variations in the stomach wall.

Values of *CW* varied from 0.1 to 4.8 g. For values of *CW* below 1.7 g, phytoplankton concentration in the stomach contents (*CP*), expressed as *a*-type phaeopigments ( $\mu\text{g}$ ), increased with increasing *CW* (Figure 1). The equation that reflects this relation was:

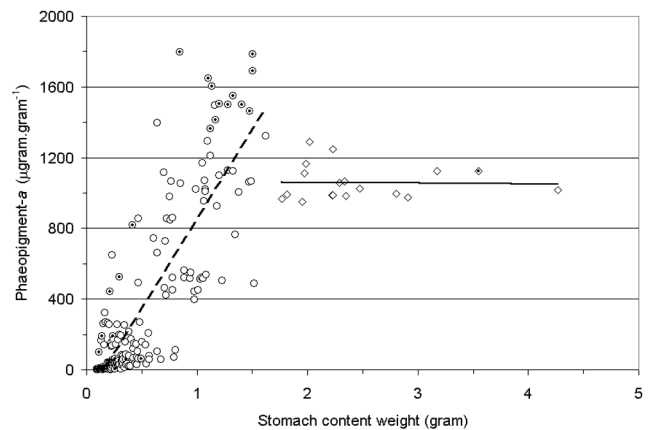
$$CP = 1002 \cdot CW - 153 (r^2 = 0.71, N = 194, P \ll 0.001) \quad (5)$$

For values of *CW* over 1.7 g, the concentrations of phaeopigments in the stomach tended to be constant with a mean of  $1100 \mu\text{g}$  per gram of content. These samples came from a single sampling station. Highest values of *CP* ( $> 1300 \mu\text{g g}^{-1}$ ) were found in a narrow range of stomach contents weights (0.7 to 1.6 g) and came also from a single sampling station.

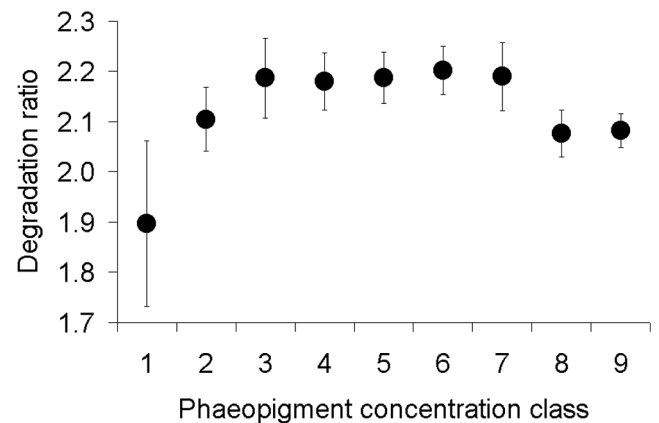
As expected, phytoplankton *a*-type pigments found in the stomachs were mostly in the form of phaeopigments. At low phaeopigment concentrations the ratio of Chl-*a* degradation (Freeman & Tukey (1950) transformed values) was relatively low and increased significantly within the first two classes (Figure 2). Degradation ratios were very high and constant at intermediate concentration levels decreasing at high concentrations.

### *Validation of the fullness scale*

From the 970 stomachs analysed 48% were almost empty (fullness 1), 37% half full (fullness 2), 12% full (fullness 3) and only 3% were bursting (fullness 4) (Table 1). No individuals were found with empty stomachs (fullness 0).



**Figure 1.** Concentration of *a*-type phaeopigments in sardine stomachs as a function of content weight. Dashed line, linear of stomach content weight  $< 1.7$  g (circles); continuous line, linear of stomach content weight  $> 1.7$  g (diamonds); inserted black dots, stomach contents with  $< 98\%$  chlorophyll-*a* degradation.



**Figure 2.** Mean ( $\pm$ SD) proportion of degradation by class of phaeopigments concentration in the stomachs (1, 1 to 199; 2, 200 to 399; 3, 400 to 599; 4, 600 to 799; 5, 800 to 999; 6, 1000 to 1199; 7, 1200 to 1299; 8, 1300 to 1599; 9, 1600 to  $1799 \mu\text{g g}^{-1}$ ). Proportion of degradation correspond to Freeman & Tukey (1950) transformed values.

**Table 1.** Percentage of paired occurrences of colour and fullness indices within the stomachs ( $N=970$ ).

Index	Fullness					Total
	0	1	2	3	4	
Colour 1	0	35.6	0.5	0.1	0	36.2
Colour 2	0	2.5	4.7	0.8	0	8.0
Colour 3	0	3.8	10.6	0	0	14.4
Colour 4	0	6.4	20.8	10.9	3.2	41.3
Total	0	48.2	36.7	11.9	3.2	100.0

Stomach mean weights and mass increased with increasing fullness categories (Table 2 and Figure 3A) with significant differences. There was a two-fold increase of stomach mass between two consecutive fullness categories with a high degree of variability particularly for the first two fullness indices.

**Table 2.** Non-transformed values of: (A) stomach content weight (g) and mass (%) by index of stomach fullness; (B) concentration of *a*-type phaeopigments ( $\mu\text{g g}^{-1}$ ) by index of stomach colour.

Scale index	Mean		SD		<i>c.v.</i>		N
	(g)	(%)	(g)	(%)	(g)	(%)	
(A)							
Fullness 0	—	—	—	—	—	—	0
Fullness 1	0.52	0.41	0.21	0.28	0.40	0.68	456
Fullness 2	0.78	0.94	0.27	0.55	0.35	0.59	356
Fullness 3	1.44	1.99	0.47	0.64	0.33	0.32	115
Fullness 4	2.78	3.87	1.01	1.08	0.36	0.28	31
(B)							
Colour 1	41.3		89.34		2.16		54
Colour 2	107.9		79.10		0.73		15
Colour 3	50.0		30.64		0.61		54
Colour 4	874.8		426.89		0.49		98

SD, standard deviation; *c.v.*, coefficient of variation ; N, number.

#### Validation of the colour scale

The majority of the examined 970 stomachs belonged to colour 4 (green) and colour 1 (beige) with 41% and 36% respectively (Table 1). Colour 2 (orange) and 3 (brown) were the least common with 8% to 14% of the stomachs.

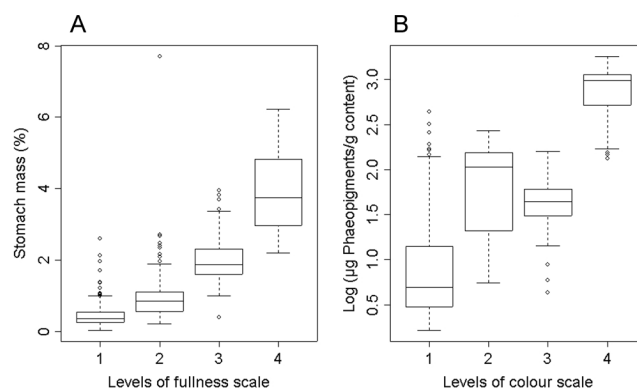
The mean concentration of *a*-type phaeopigments ( $\mu\text{g}$ ) per gram of content in each colour index and the respective standard deviations (Table 2) were determined for a smaller set of stomachs (N=221). Non-transformed mean concentrations of phaeopigments varied more than 20 times between the lowest values at colour 1 and the highest at colour 4. These two indices were also the categories that presented, respectively, the highest and the lowest degrees of variability as indicated by the *c.v.*

With the exception of colour categories 2 and 3 phaeopigment concentrations in the stomachs were significantly different from each other (Figure 3B). The merging of categories 2 and 3 led to a mean value of  $61.9 \mu\text{g g}^{-1}$  of *a*-type phaeopigment concentration with a standard deviation of 51.1. Although it had a higher *c.v.*, the grouped mean was statistically different from levels 1 and 4.

When comparing prey volumes by weight in each colour category (Table 3), colour 2, with  $270.3 \text{ mm}^3 \text{ g}^{-1}$ , was the bulkiest, followed by colours 3 and 4, with respectively  $177.3$  and  $148.7 \text{ mm}^3 \text{ g}^{-1}$ . Colour 1, with only  $43.0 \text{ mm}^3 \text{ g}^{-1}$ , had the lowest plankton volume per gram of content. Zooplankton volumes were higher in colours 2 and 3 while colour 4 contained the highest volume of phytoplankton. Relative abundance, in volume, of prey in the stomach per colour category showed that proportions of phyto- to zooplankton were similar in colours 2 and 3 while colour 4 had the highest percentage of phytoplankton (19%).

#### Relation of colour and fullness scales

Fullness indices had significantly different concentrations of phaeopigments ( $\mu\text{g g}^{-1}$ ) in the stomachs and the concentration increased with fullness category. Only fullness 3 and 4, with phaeopigment concentration means of respectively



**Figure 3.** Box-and-whisker plots of: (A) stomach content mass in each category of the fullness scale; (B) log transformed concentration of *a*-type phaeopigments on the stomach content by colour scale category. (Boxes stretch from a lower hinge (25% percentile) to an upper hinge (75% percentile) and are crossed by a horizontal line (the median). Whiskers limit the smallest and largest values. Open circles correspond to outliers).

$1,033$  and  $1,175 \mu\text{g g}^{-1}$  were not significantly different from each other. Mean phaeopigment-*a* concentration values were  $61 \mu\text{g g}^{-1}$  for fullness 1 and  $250 \mu\text{g g}^{-1}$  for fullness 2.

With exception of colours 2 and 3 the colour indices had also significantly different stomach mass. These values varied between 0.28% of the gutted weight for colour 1 to 2.03% for colour 4, with colours 2 and 3 containing respectively 0.91% and 0.78%.

The joint effect of the above results is reflected in the paired occurrences of colour and stomach fullness categories (Table 1) with some colour indices appearing preferentially on some levels of fullness. A  $\chi^2=526.57$  led to the rejection of  $H_0$  at  $P<0.001$ , meaning that the distribution of the fullness categories is not independent of the colour levels. Most of the stomachs displayed colour and fullness indices belonging to category 1 (36%) followed by the pairing of colour 4 and fullness 2 (21%). Colour 4 was the only colour in fullness 4. The least observed colours, 2 and 3, were most abundant in fullness 2.

**Table 3.** Volume of prey taxa per gram of stomach content ( $\text{mm}^3 \text{g}^{-1}$ ) in each colour category and size fraction. Second column (Mean vol.) is the average volume of each individual prey ( $\text{mm}^3$ ). The last three rows are the proportions (%) of the different plankton groups.

Taxa/Species	Mean vol.	Colour 1		Colour 2		Colour 3		Colour 4	
		> 200 $\mu\text{m}$	< 200 $\mu\text{m}$	> 200 $\mu\text{m}$	< 200 $\mu\text{m}$	> 200 $\mu\text{m}$	< 200 $\mu\text{m}$	> 200 $\mu\text{m}$	< 200 $\mu\text{m}$
<b>PHYTOPLANKTON</b>									
Dinophyceae n.i.	$2.3 \times 10^{-4}$	—	$4.4 \times 10^{-1}$	—	$3.2 \times 10^{-1}$	—	$8.7 \times 10^{-1}$	—	3.4
<i>Ceratium</i> spp.	$2.6 \times 10^{-4}$	—	—	—	$4.4 \times 10^{-2}$	—	—	—	$8.5 \times 10^{-1}$
<i>Dinophysis</i> spp.	$2.8 \times 10^{-4}$	—	$2.0 \times 10^{-2}$	—	$2.4 \times 10^{-1}$	—	$5.3 \times 10^{-2}$	—	—
<i>Prorocentrum micans</i>	$5.8 \times 10^{-5}$	—	$8.0 \times 10^{-3}$	—	—	—	—	—	—
<i>Protoperdinium</i> spp.	$2.3 \times 10^{-4}$	—	$1.3 \times 10^{-1}$	—	$4.1 \times 10^{-2}$	—	$2.9 \times 10^{-1}$	—	3.0
<i>Scipsiella</i> spp.	$1.4 \times 10^{-5}$	—	$7.0 \times 10^{-2}$	—	—	—	$3.9 \times 10^{-3}$	—	$1.2 \times 10^{-2}$
Cyst	$4.0 \times 10^{-5}$	—	—	—	—	—	—	—	$1.2 \times 10^{-2}$
Centric diatoms n.i.	$4.1 \times 10^{-5}$	—	—	—	$2.1 \times 10^{-2}$	—	—	—	$2.5 \times 10^{-2}$
<i>Bidulphia alternans</i>	$3.5 \times 10^{-5}$	—	$2.5 \times 10^{-3}$	—	—	—	—	—	$1.1 \times 10^{-2}$
<i>Coscinodiscus</i> spp.	$1.8 \times 10^{-4}$	—	$7.4 \times 10^{-2}$	—	—	—	$5.0 \times 10^{-2}$	—	—
<i>Diploneis</i> spp.	$4.8 \times 10^{-5}$	—	—	—	$8.3 \times 10^{-3}$	—	$9.1 \times 10^{-3}$	—	—
<i>Paralia sulcata</i>	$1.2 \times 10^{-6}$	—	—	—	—	—	$4.5 \times 10^{-3}$	—	$1.3 \times 10^{-2}$
<i>Pseudo-nitzschia</i> spp.	$2.6 \times 10^{-6}$	—	$1.8 \times 10^{-4}$	—	—	—	—	—	$2.1 \times 10^1$
<i>Thalassiosira</i> spp.	$2.1 \times 10^{-5}$	—	$8.9 \times 10^{-3}$	—	$3.7 \times 10^{-3}$	—	—	—	—
<i>Thalassionema</i> spp.	$8.0 \times 10^{-7}$	—	$3.3 \times 10^{-4}$	—	—	—	—	—	—
<b>ZOOPLANKTON</b>									
Tintinnoinea	$2.6 \times 10^{-4}$	—	$3.6 \times 10^{-2}$	—	$8.8 \times 10^{-2}$	—	$4.9 \times 10^{-2}$	—	—
Siphonophora	$6.7 \times 10^{-1}$	—	—	—	—	—	$1.7 \times 10^1$	—	—
Foraminifera	$1.7 \times 10^{-2}$	—	—	$2.4 \times 10^{-1}$	—	—	—	—	—
Lamelibranchiata (veligers)	$9.4 \times 10^{-3}$	—	—	—	—	—	$8.2 \times 10^{-1}$	—	—
Crustacea (eggs)	$2.6 \times 10^{-4}$	$5.3 \times 10^{-4}$	$3.7 \times 10^{-1}$	$6.1 \times 10^{-1}$	$3.6 \times 10^{-1}$	—	$2.8 \times 10^{-1}$	—	2.9
Copepoda (nauplii)	$1.8 \times 10^{-3}$	—	$3.7 \times 10^{-1}$	—	$3.0 \times 10^{-1}$	—	$3.3 \times 10^{-1}$	—	—
<i>Euterpina acutifrons</i>	$2.4 \times 10^{-3}$	$4.1 \times 10^{-1}$	—	2.2	—	$1.3 \times 10^1$	2.1	$4.4 \times 10^{-1}$	—
<i>Oncaea</i> spp.	$2.4 \times 10^{-2}$	$3.2 \times 10^{-1}$	—	1.2	2.5	$5.6 \times 10^{-1}$	—	$7.3 \times 10^{-2}$	—
<i>Corycaeus</i> spp.	$2.4 \times 10^{-2}$	$6.5 \times 10^{-2}$	—	4.1	—	3.3	—	—	—
Calanoida n.i.	$2.9 \times 10^{-1}$	1.6	—	$2.1 \times 10^1$	—	8.2	—	—	—
<i>Temora longicornis</i>	$5.8 \times 10^{-2}$	$1.7 \times 10^{-1}$	—	$2.9 \times 10^1$	—	$5.6 \times 10^1$	—	$8.6 \times 10^{-1}$	—
<i>Centropages chierchiae</i>	$2.9 \times 10^{-1}$	—	—	$4.5 \times 10^1$	—	$2.2 \times 10^1$	—	$9.6 \times 10^{-1}$	—
<i>Acartia clausi</i>	$5.8 \times 10^{-2}$	$1.5 \times 10^{-1}$	—	3.0	—	1.4	—	1.1	—
<i>Pleurommama gracilis</i>	$2.9 \times 10^{-1}$	—	—	$8.1 \times 10^1$	—	—	—	—	—
<i>Candacia armata</i>	$2.9 \times 10^{-1}$	—	—	—	—	7.0	—	—	—
Cirripedia (nauplii)	$9.4 \times 10^{-3}$	—	—	$5.1 \times 10^{-1}$	—	3.4	—	$1.9 \times 10^{-2}$	—
Cirripedia (cypris)	$3.1 \times 10^{-2}$	$6.3 \times 10^{-2}$	—	$8.8 \times 10^{-1}$	—	1.0	—	—	—
Brachiura (larvae)	$5.5 \times 10^{-2}$	—	—	1.5	—	$3.3 \times 10^{-1}$	—	—	—
Fish eggs	$5.4 \times 10^{-1}$	$3.8 \times 10^1$	—	$7.5 \times 10^1$	—	$3.8 \times 10^1$	—	$1.9 \times 10^{-2}$	—
<b>OTHERS</b>									
Mucilaginous cyst	$2.7 \times 10^{-4}$	—	$5.6 \times 10^{-1}$	—	$2.3 \times 10^{-1}$	—	$2.6 \times 10^{-2}$	—	$2.4 \times 10^{-1}$
Pollen grains	$1.1 \times 10^{-3}$	—	—	—	1.5	—	$5.1 \times 10^{-1}$	—	$3.3 \times 10^{-1}$
TOTAL		40.95	2.09	264.65	5.70	172.74	4.60	117.40	31.34
PHYTOPLANKTON			1.8		0.3		0.7		18.8
ZOOPLANKTON			96.9		99.1		99.0		80.9
OTHERS			1.3		0.6		0.3		0.4

## DISCUSSION

Most clupeids are microphages capable of particulate and filter-feeding on a wide range of particle sizes. In the case of sardines, food intake seems to be controlled mainly by the filtering rate and the concentration of food in the water. Stomach content weights and composition reflect the food intake although they are obviously influenced by type and concentration of food as well as by the degree of digestion and gut clearance. In the present study, the most important constituent of the diet, in volume, were zooplankters as indicated by the prey analyses of the contents (Table 3). Even when the highest proportion of phytoplankton was found, zooplankton volumes account for more than 80% of the volume of the content, which highlight their importance in the diet of adult sardines

and support the results of van der Lingen (1998a), Garrido (2003) and Bode et al. (2003, 2004). The plankton taxa composition on the stomachs seems to reflect the most frequent groups of the Portuguese waters (Cunha, 2002) which may indicate a non selective type of feeding. Most of the phytoplankton pigments in the stomachs were in the form of *a*-type phaeopigments due to degradation of Chl-*a* by gastric acidification. The highest values of phaeopigment-*a* concentrations in the stomachs ( $>1,400 \mu\text{g g}^{-1}$ ) were associated with relatively lower percentages of degradation (Figure 2), indicating that the phytoplankton had been ingested recently, before gastric juices had time to degrade completely the Chl-*a*. Results show that stomachs with contents below 1.7 g exhibited a linear relationship between the weight of the contents and the concentration of phytoplankton, suggesting either filter-feeding at a rate

proportional to food concentration or an inverse proportionality to digestion rate. The fact that stomach contents with low concentrations of *a*-type phaeopigments ( $<400 \mu\text{g g}^{-1}$ ) have the lowest mean proportions of degradation (Figure 2) precludes the latter hypothesis. The highest filtering rates must have occurred when stomach contents weighed between 0.7 and 1.7 g, i.e. when the highest values of phytoplankton concentration in the stomachs with significantly low proportions of degradation were found. Above weights of 1.7 g, the concentration of phytoplankton in the stomach contents remained constant, suggesting that the filtering rate is inversely proportional to food concentration or that sardines were mainly particulate feeding on zooplankton. However, these hypotheses should be taken cautiously since those stomachs came from a single station.

Although the data collected by using the fullness and colour scales were not as detailed as those obtained by measuring stomach weights and microscopically examining the prey, the results obtained were comparable. With respect to the fullness scale, the categories seemed to reproduce quite well the amount of food intake by sardines as reflected by the stomach mass. The categories for the colour scale reflect the amount and type of plankton eaten by sardines as given by the concentration of *a*-type phaeopigments in the stomachs and the microscopic analysis of their contents, which make it a good indicator for ecological studies involving the relation of sardine distributions during spawning and resting periods to food availability. As was expected, colour 4 (green) had the largest concentration of Chl-*a* and volume of phytoplankton cells. Colours 2 and 3 (orange and brown, respectively) reflect stomach contents with highest volume of zooplankton per gram of content ( $\approx 99\%$ ). Differences in mean concentrations of *a*-type phaeopigments and proportions of degradation of Chl-*a* between colours 2 and 3 were not statistically significant. Difference in colour of these two categories may reflect slightly different zooplankton species composition or/and a different degree of digestion. Therefore there is no reason to keep these two colour categories distinct and they should be merged. Beige stomachs (colour 1) were the least voluminous both in phytoplankton and zooplankton items (Table 3) per gram of stomach content which probably reflect a high degree of digestion. The calibration of sardine diet using the scales presented in this work was performed in sardines of a wide length range (8.7–23.2 cm), leading us to propose that these indices can be used for the entire juvenile and adult sardine population.

Based on the colour and fullness categories of the stomachs it is possible that the majority of stomachs were almost empty (fullness 1) or at most half-full (fullness 2). As indicated by the colour indices, 22.4% of the sardines had a diet composed mainly of zooplankton (colours 2 and 3), corresponding to 90% of prey biovolume, according to prey analysis, while for 41.3% phytoplankton (colour 4) was also important, representing approximately 20% of total prey biovolume. The stomachs were in an advanced degree of digestion or almost empty (colour 1) in 36.2% of the sardines.

In general, almost empty stomachs (fullness 1) were beige (colour 1), which probably reflects the colour of the muscle. Half-full (fullness 2) stomachs included a large variety of colour categories (Table 1), although green

(colour 4) was present in high numbers. Full (fullness 3) and bursting (fullness 4) stomachs were mostly green. No empty stomachs (level 0) were registered among the almost 1000 stomachs analysed suggesting that this category rarely occurs and that it should be dropped from the fullness scale. The fact that no empty stomachs were found might be an indication either that sardines have gut clearance rates proportional to the amount of ingested food, or that they were always feeding as found by van der Lingen, (1998a) for *Sardinops sagax*.

After revision of the initial scales the following are suggested as reliable estimators of feeding intensity and quality in sardine:

Colour scale: 1, beige; 2, orange + brown; 3, green.

Fullness scale: 1, almost empty; 2, half-full; 3, full; 4, bursting.

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