



# Diel feeding intensity and daily ration of the sardine *Sardina pilchardus*

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**ABSTRACT:** The diel variation in feeding intensity and daily ration of the sardine *Sardina pilchardus* (Walbaum, 1792) was investigated based on 50 pelagic trawl hauls carried out during the period of thermal stratification (July 2007 and July 2008) and mixing (December 2007 and February 2009) in a coastal area of the North Aegean Sea (eastern Mediterranean). Concurrently collected hydrographic (temperature and salinity), plankton (chlorophyll *a*, microzooplankton and mesozooplankton) and fish parameters (somatic condition, sexual maturity and catch per unit effort of sardine, CPUE) were used to explain seasonal and inter-annual variability in feeding periodicity and daily ration. A piecewise regression fitted to the weight-length data indicated a significant inflexion point at a length of ~100 mm that was used to split the fish sample into adults and juveniles. The stomach fullness index (stomach content mass/fish mass) was strongly dependent on size, and a generalized linear model was used instead to standardize stomach content mass. During summer, sardine fed consistently during daytime with a prominent peak at around sunset. In winter, high feeding rates were recorded in the early night (first 6 h after sunset) but not in the second half of the night. Field estimates of gastric evacuation rate ( $R$ ) ranged from 0.101 to 0.225 h<sup>-1</sup> and were strongly related to temperature ( $T$ ) ( $R = 0.075e^{0.038T}$ ,  $r^2 = 0.785$ ). Daily rations were estimated by applying the Elliott-Persson and the Eggers models and varied from 2.02 to 3.67% total weight (TW) in adults and 4.18 to 5.36% TW in juveniles. A significant positive relationship emerged when daily ration was regressed against the ratio of mesozooplankton biomass to sardine CPUE, implying a density-dependent rate of food consumption.

**KEY WORDS:** Daily ration · Feeding periodicity · Sardine · *Sardina pilchardus* · Mediterranean Sea · Aegean Sea

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## INTRODUCTION

Small pelagic fish such as herrings, sardines and anchovies are of great economic importance, comprising approximately 30% of the global fish catch (FAO 2008). Their high abundance in the World Ocean is mainly attributed to their ability to exploit lower trophic levels, i.e. plankton, very efficiently (van der Lingen et al. 2009), especially in highly productive, upwelling systems. They are a key component of the systems where they are present (Freon et

al. 2009), channeling the energy from low to high trophic levels (van der Lingen et al. 2009). In most ecosystems, sardines are primarily non-selective filter feeders (van der Lingen et al. 2009), although in laboratory studies they have been observed to exhibit prey size or prey type selectivity, particularly when particulate feeding (van der Lingen 1994, Garrido et al. 2007).

Food consumption in fish is considered to depend primarily on body size and water temperature (Jobling 1998). Consumption increases with body size fol-

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lowing a power function and is usually expressed as a proportion of body weight consumed within a diel cycle (daily ration: grams of food consumed per gram of fish weight per day) (Jobling 1998). The response of consumption to water temperature is dome-shaped; feeding rates increase as temperature rises, reach a maximum at some intermediate temperature and further temperature increase leads to a drastic reduction in feeding rates (Jobling 1998). Recent attempts to couple small pelagic fish bioenergetics models with lower trophic level models (LTLs) have generally considered this size and temperature dependency of plankton consumption (e.g. Politikos et al. 2011). However, other factors may additionally affect food consumption (e.g. photoperiod, reproductive activity, previous days' consumption), but have been poorly investigated in fish (Jobling 1998 and references therein).

Sardine stocks (*Sardina pilchardus* and *Sardinops sagax*) are generally found in productive coastal upwelling regions along the eastern margins of the Atlantic and Pacific Oceans (Freon et al. 2009). In contrast, the Mediterranean Sea is one of the most oligotrophic areas of the world, with very low nutrient concentrations (Azov 1991) and intense thermal stratification during summer. In this basin, small pelagic fish stocks, including the sardine *Sardina pilchardus*, inhabit coastal areas typically associated with less saline waters (e.g. Ebro River delta in the Catalan Sea, Gulf of Lions, Po River in the Adriatic Sea, North Aegean Sea) which are characterized by relatively higher productivity (Somarakis et al. 2006). In recently developed trophic mass-balance models for the Mediterranean Sea, the importance of sardines has been highlighted in all systems considered, namely the Catalan Sea, the Adriatic Sea and the NE Aegean Sea (Tsagarakis et al. 2010).

Information on the diel variation and intensity of feeding of sardine in the Mediterranean is scarce and contradictory. Andreu (1969) suggests that sardines feed during daylight, but Gómez-Larrañeta (1960) claims that feeding continues throughout the night. In upwelling regions, sardines are considered continuous or daytime feeders (van der Lingen et al. 2008 and references therein). The main objective of the present study was to assess the pattern of diel periodicity, feeding intensity and consumption of sardines and, where possible, the principal characteristics of the water column that might control this pattern. The analysis was based on fish samples collected at a coastal site in the NE Aegean Sea (eastern Mediterranean) that covered the entire 24 h cycle in both summer (July 2007 and July 2008) and winter (December 2007 and February 2009). In particular, we were interested in answering the following questions: (1) Is there any peak in the feeding intensity of sardines or fish fed continuously throughout the diel cycle; (2) Does the daily ration change with ontogeny; (3) Does the daily ration differ between the reproductive (winter; Ganias et al. 2007) and growing/energy storage season (summer; Ganias et al. 2007); and (4) Do prey availability and/or physical conditions affect consumption rates?

## MATERIALS AND METHODS

### Data collection

Sardines *Sardina pilchardus* were sampled at a shallow (mean depth  $\approx 30$  m), coastal site in the North Aegean Sea (Fig. 1) in the vicinity of the mouth of the Nestos River. Sampling took place onboard the 25 m RV 'Philia' in July 2007 (S07), December 2007 (W07), July 2008 (S08) and February 2009 (W09). A small pelagic trawl with a 7 m vertical opening and 8 mm mesh size (knot-to-knot) in the cod-end was used. Sardines were captured in a total of 50 hauls. Tow duration was generally shorter than 30 min, to minimize stress and cod-end feeding of fish. Trawling depth was adjusted according to fish position in the water column, and it was variable during the day (the trawl was towed in a step-oblique manner) in order to catch both adults (distributed close to the bottom) and juveniles (more surface oriented) (Anonymous 2010). Trawling was carried out close to the surface at night, because sardines migrate to this layer at dusk where schools disperse (Giannoulaki et al. 1999). Given constraints in sampling over the 24 h onboard the small research vessel, trawling was car-

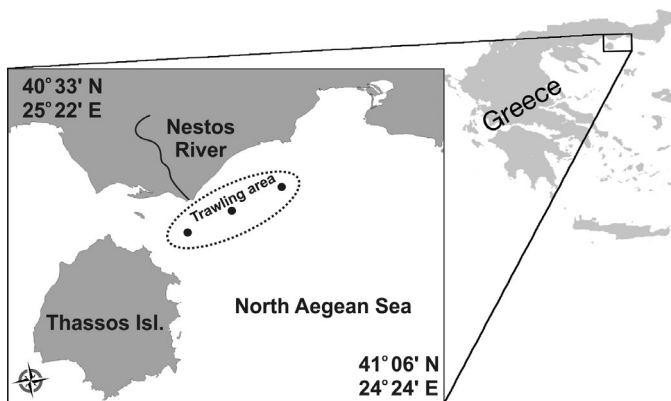


Fig. 1. Map of the study area indicating the trawling area and the 3 sampling stations where environmental parameters were collected

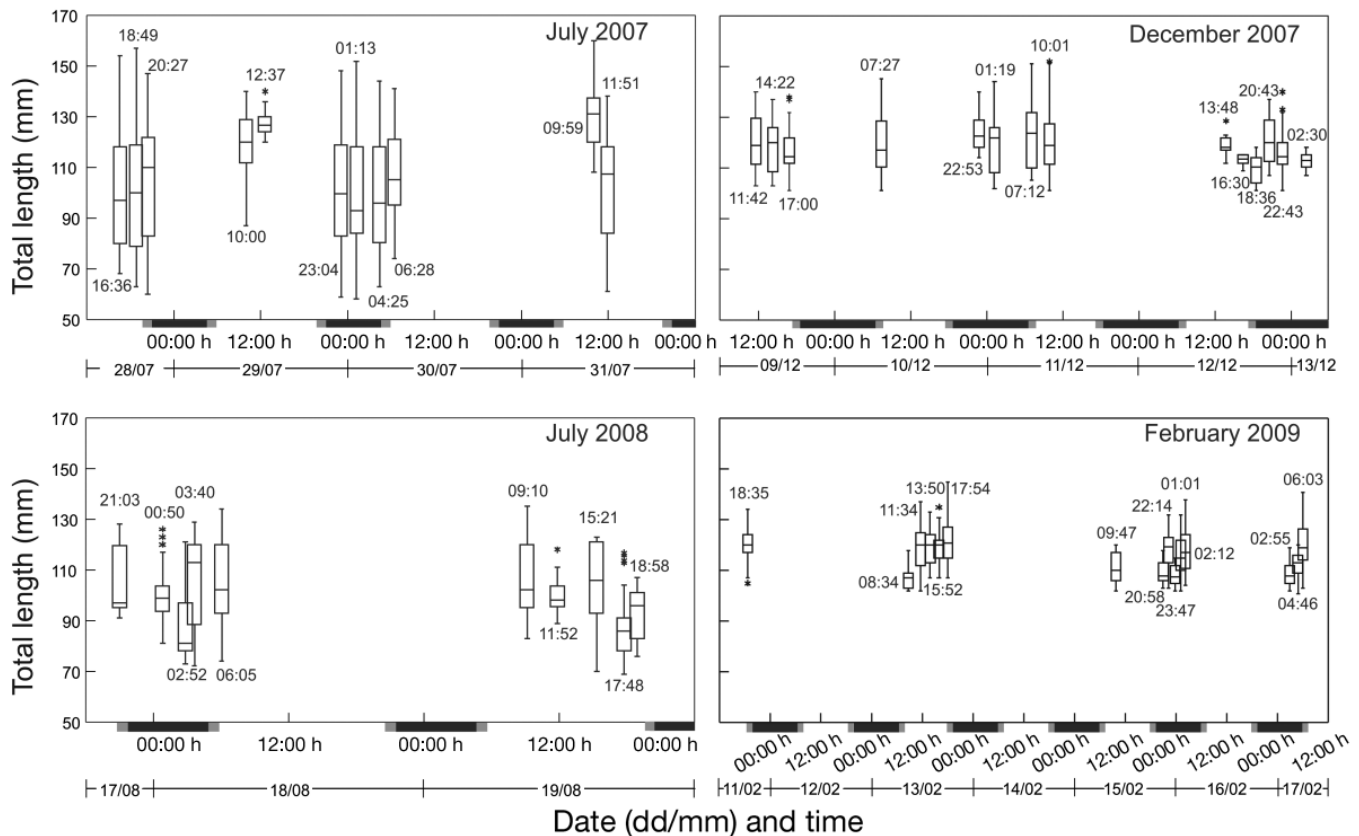


Fig. 2. *Sardina pilchardus*. Boxplots of sardine length for all hauls performed in each sampling period. Boxes represent interquartile ranges; internal horizontal bars represent the median; whiskers represent 1.5× the interquartile range, and asterisks are given for outliers. Mean sampling time is also indicated. Nautical twilight (grey bars) and nighttime (black bars) are also shown on the bottom axes

ried out at different times of day on consecutive days in an attempt to cover the entire 24 h cycle (Fig. 2). Occasionally, sampling was not possible due to bad sea conditions or the catch was negative for sardine. Hence, in order to examine the daily ration and feeding periodicity, samples from different dates (Fig. 2) were pooled and assigned to 8 consecutive 3 h time intervals representing an entire diel cycle (i.e. 23:00 to 02:00, 02:00 to 05:00, ..., 20:00 to 23:00 h).

A SBE 25 CTD profiler was used in each sampling period to record temperature, salinity and fluorescence at 3 stations covering the mean trawling path (Fig. 1). Mesozooplankton and microplankton samples were collected with vertical tows of a standard WP2 net (200  $\mu\text{m}$  mesh size) and a scaled-down version (25 cm diameter) of the same sampler (50  $\mu\text{m}$  mesh size). Only the size fractions from 50 to 200  $\mu\text{m}$  and from 200 to 2000  $\mu\text{m}$  were used for biomass estimations of microplankton and mesozooplankton, respectively, after sieving each sample through appropriate meshes to exclude organisms >200  $\mu\text{m}$  and >2000  $\mu\text{m}$ . Each fraction was filtered on pre-weighed

GF/C filters and dried at 60°C to constant weight. Depth-stratified water samples were also collected for chlorophyll *a* (chl *a*) determination.

After each haul, fish were immediately put in iced seawater, sorted into 4 size classes (40–80, 81–100, 101–120 and >120 mm) and at least 20 fish per size class (when possible) were frozen at –35°C. Two size classes were finally considered for analysis—juveniles (<100 mm) and adults ( $\geq$ 100 mm)—after merging the 40–80/81–100 and 101–120/>120 mm size classes due to insufficient numbers of fish in the original size class in each 3 h time interval (see ‘Results’).

In the laboratory, fish were thawed, and measurements of total length (TL, mm), total weight (TW, 0.01 g) and eviscerated weight (EW, 0.01 g) were taken. Gender, macroscopic maturity and gonad weight (GW, 0.1 mg) were also recorded. The digestive tracts were removed, stomachs were dissected and their contents were washed with distilled water, filtered on pre-weighed Whatman GF/C filters and dried at 60°C to constant weight. Only contents of the stomach (cardiac, cecum and pyloric part) were ana-

lyzed. Contents of the intestine were discarded to reduce bias caused by different rates of digestion and gut passage times (Hyslop 1980). Dry weight (DW) of stomach contents was considered in the present study. No signs of regurgitation due to sampling stress were recorded.

In addition to fish used in the stomach content analysis, additional specimens were collected randomly from the trawl catches and transferred to the laboratory for analyses. These random samples were pooled with those used in the stomach content analysis to calculate weight–length relationships for each sampling period.

### Data analysis

The EW–length (TL) relationship of sardine ( $n = 3041$ ) caught and measured during the summer cruises was examined first to assess whether a shift in allometric growth existed that could be used to separate the juvenile from the adult stage (Nikolioudakis et al. 2010). For this purpose, a piecewise regression was fit to the log-transformed data (natural logarithms):

$$\log(\text{EW}) = b_0 + b_1 \log(\text{TL}) + b_2 \{[\log(\text{TL}) - b_3] \times [\log(\text{TL}) \geq b_3]\} \quad (1)$$

where  $b_0$  is the  $y$ -intercept,  $b_1$  is the slope of the regression during the juvenile stage,  $b_2$  is the change in slope that results in the slope of the relationship during the adult stage, and  $b_3$  is the mean length at change from juvenile to adult (Nikolioudakis et al. 2010).

The average catch per unit effort (CPUE) of sardine (catch [kg]/haul duration [h]) in the hauls that had been carried out during the night was calculated as an index of sardine density in the sampling area. Nighttime hauls were considered more representative than the daytime hauls given the diel variation in aggregation patterns, vertical distribution of juveniles and adults and changing trawling depth (see above) during the day (Wilson et al. 1996).

The mean somatic condition (SC) of adult or juvenile fish in each sampling period (SP) was estimated from an analysis of covariance model (ANCOVA) (Somarakis et al. 2011):

$$\log(\text{SC}) = a + b_1 \log(\text{TL}) + b_2(\text{SP}) + b_3 \log(\text{TL})(\text{SP}) \quad (2)$$

Estimated marginal means in each sampling period at mean  $\log(\text{TL})$  represented the somatic condition (Somarakis et al. 2011). Multiple comparisons of somatic condition were made after Bonferroni adjust-

ments of significance levels. Differences in environmental parameters between periods were tested by ANOVAs or, when assumptions of ANOVA were not met, by Kruskal-Wallis tests. Student-Newman-Keul's or Dunn's tests were used for parametric and non-parametric multiple comparisons (Zar 1999).

A total of 1934 stomachs were used in the daily ration analysis. Empty stomachs were not considered, as recommended by Bromley (1994). For each fish, a stomach fullness index (SFI) was calculated by dividing the stomach content DW (measured to the nearest 0.1 mg) by EW [SFI = DW  $\times$  1000/EW]. SFI (and various modifications of it) is an index that has been used widely in studies of daily ration (e.g. Tudela & Palomera 1995, H eroux & Magnan 1996, van der Lingen 1998, Plounevez & Champalbert 2000). However, when the index was applied to sardines, a strong size-dependency was revealed (see 'Results'). Hence, we did not use this index in any subsequent analysis. Values of stomach content DW were used instead after adjusting for the effect of fish size using generalized linear models (GLMs; McCullagh & Nelder 1989). The predictor variables of GLMs were SP, sampling time (Time), fish EW and, in the case of adults, gender (G). The most appropriate link function and error model were chosen on the basis of residual plots. A log-link function along with a gamma error structure model was found to be adequate. Modeling was carried out using the R software (R Development Core Team 2009). All predictors and their first and second order interactions were initially included in the model. The stepAIC function (R package 'MASS', V7.3-5; Venables & Ripley 2002) was used to select the significant predictors and to estimate the coefficients of the models. Predictors were removed by backward elimination based on Akaike's information criterion (AIC). AIC balanced the degree of fit of a model with the number of variables, in order to find the most parsimonious model. Only those predictors which contributed significantly to the model were kept. Estimated marginal means of DW (fixed at mean EW) for each sampling period and time interval ('effects' package; Fox 2003) were used as inputs to the consumption models described below.

For estimating daily ration, 2 widely used consumption models were considered: (1) the Elliott & Persson (E-P) model (Elliott & Persson 1978) and (2) the Eggers model (Eggers 1979). In the Eggers model, feeding is considered continuous and constant during the feeding period ( $T_i$ ), while in the E-P model feeding is assumed to be constant only within each specific, consecutive sampling interval. Both models are based on the assumption that stomach contents

decline exponentially with time. Moreover, to perform accurately, the E-P model requires that sampling intervals be 3 h or shorter (Elliott & Persson 1978).

For the E-P model, consumption ( $C_t$ ) for each time interval ( $t = 3$  h) was estimated as:

$$C_t = [(S_t - S_0 e^{-R \times t}) R \times t] / (1 - e^{-R \times t}) \quad (3)$$

where  $S_0$  and  $S_t$  are the standardized stomach content at the beginning and end of each time interval  $t$  and  $R$  is the instantaneous gastric evacuation rate. The daily ration ( $C_{E-P}$ , daily ration) was calculated as the sum of partial consumptions over the assumed  $T_f$ . The feeding period was defined as the period from minimum to maximum standardized stomach content (Tudela & Palomera 1995). For the Eggers model, the daily ration was estimated as:

$$C_E = S_{avg} \times R \times T_f + (S_b - S_e) \quad (4)$$

where  $S_{avg}$  is the average stomach content DW over  $T_f$ , and  $S_e$  and  $S_b$  are the stomach content DW at the end and at the beginning of  $T_f$ , respectively. This model assumes that the weight of the stomach contents changes significantly between the beginning and end of  $T_f$ . The standard error for  $C_{E-P}$  was approximated following the  $\delta$  method as proposed by Worobec (1984).

The instantaneous gastric evacuation rate ( $R$ ) was estimated as recommended in Héroux & Magnan (1996): for all pairs of consecutive hauls performed during the same day a series of  $R$  values were calculated:

$$R = (\ln S_t - \ln S_0) / t_i \quad (5)$$

where  $t_i$  is the time elapsed between hauls. The gastric evacuation rate used was the maximum  $R$  ( $R_{max}$ ).  $R$  was estimated for each sampling period separately for adults and juveniles. The assumptions for using  $R_{max}$  were that stomach contents declined exponentially during the non-feeding period and that no feeding was taking place at that time.

For comparison with results of similar studies, daily ration was also calculated as a fraction of total wet weight (%TW) by converting DW to wet weight. For this conversion, we assumed a water content value of 87%, i.e. that of copepods of the genus *Acartia* (Borme 2006). Copepods are the most significant part of the diet (in terms of weight and carbon contribution) for the Mediterranean sardine (authors' unpubl. data, but see e.g. Garrido et al. 2008 for the Iberian sardine).

Finally, estimates of consumption in different sampling periods were compared with environmental

and fish parameters recorded during the surveys using appropriate regression and/or ANCOVA models. The density dependency of consumption was evaluated by comparing it with the ratio of mesozooplankton biomass to sardine CPUE.

## RESULTS

The residual plot of the weight-length relationship of fish *Sardina pilchardus* caught in summer (Fig. 3A) revealed a non-random (structured) distribution of residuals indicative of an ontogenetic transition (Nikolioudakis et al. 2010 and references therein). The piecewise regression fitted to the weight-length data [ $\log EW = -12.12 + 3.05 \times \log TL + 0.343 \times (\log TL - 4.62) \times (\log TL \geq 4.62)$ ],  $r^2 = 0.988$ ,  $p < 0.0001$ ,  $n = 3041$ ) had a significant ( $p < 0.0001$ ) inflexion point at the length of 101.43 mm TL (95% confidence intervals: 99.03 to 103.90 mm) (Fig. 3B) and randomly distributed residuals. Fish size range in each haul is shown in Fig. 2. During the winter cruises, all sardines were  $>100$  mm (Fig. 2), with their gonads classified macroscopically as developing/mature (see also mean gonadosomatic index [GSI] values in Table 1). The weight-length relationship for winter had randomly distributed residuals (not shown).

The water column was well stratified in summers and mixed in winters (Fig. 4), with significantly higher temperatures and lower surface salinities in S07 and S08 (Table 1). Productivity was higher in winters as indicated by the increased chl *a*, microplankton and mesozooplankton, with mesozooplankton biomass being significantly higher only in W07 (Table 1). Sardine CPUE was higher in 2007, especially in S07. The somatic condition of adults was lower in winter, when GSIs were higher (sardine reproductive period) (Table 1).

Plots of the SFI against fish weight revealed that the SFI was often size dependent, especially in summer when both juvenile and adult sardines were sampled (e.g. July 2007; Fig. 5). Hence, use of the SFI was not appropriate for comparisons of samples with different fish sizes. We therefore adopted the GLM approach to standardize stomach contents for the effect of fish size.

The most parsimonious GLMs for juveniles ( $<100$  mm) and adults ( $\geq 100$  mm) explained 91.12 and 75.05% of the deviance, respectively (Table 2). In both cases, Time was the predictor that explained the largest proportion of deviance ( $\sim 77\%$  for juveniles and  $\sim 42\%$  for adults). Significant interactions of Cruise and Time with fish EW were interpretable in



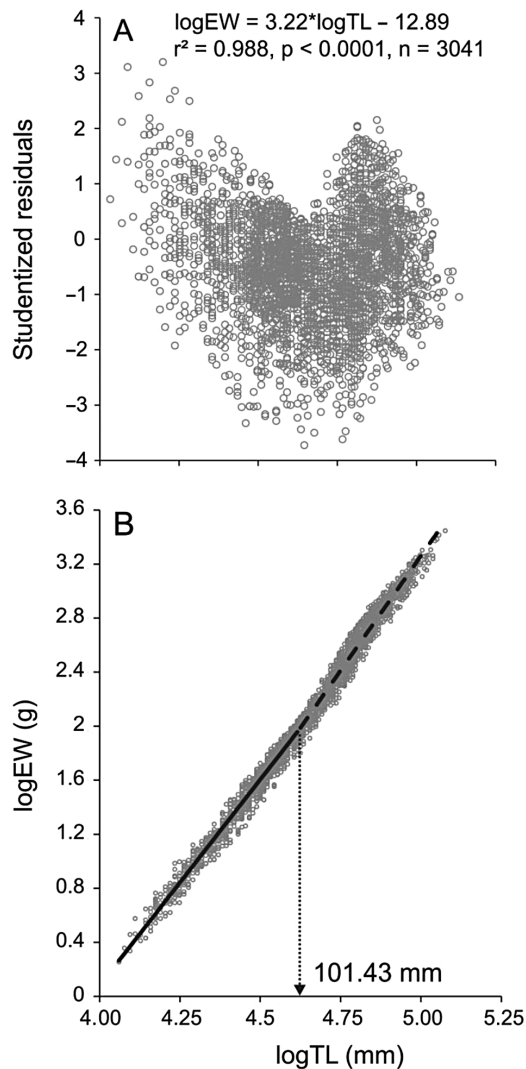


Fig. 3. *Sardinia pilchardus*. (A) Plot of the residuals of the single-stage, weight–length relationship of sardines captured during summers. (B) Piecewise regression fitted to the same dataset. The estimated inflection point (length at slope change) is indicated. EW: eviscerated weight; TL: total length

terms of changing slopes of the DW–EW relationship between cruises and time intervals (see also Fig. 4). With regards to adults, terms including gender (G) were not significant.

The estimated marginal means with 95% confidence intervals for the multiplicative effect of Cruise  $\times$  Time are shown in Fig. 6. Fish EW was fixed at 4.43 g for juveniles and 11.58 g for adults. A clear daytime feeding pattern was revealed in summer for both juveniles and adults. The lowest DWs occurred around sunrise, progressively increasing throughout the course of the day, to peak at around sunset (Fig. 6A,B,C,E). All stomachs analyzed in July were full at sunset, and prey items were intact. In winter, sardines were feeding throughout the day and continued to feed in the early night (Fig. 6D,F), which contrasted with summer, when feeding ceased at night. After the peak feeding time (during dusk in summer and after midnight in winter), the stomach DW dropped (Fig. 6). The degree of digestion of prey items in stomachs increased as the night progressed further, implying that the non-feeding period took place during the night.

The estimated gastric evacuation rates are given in Table 3. *R*-values of juveniles and adults in summers were similar. A significant relationship existed be-

Table 1. *Sardinia pilchardus*. Comparison of physical, biotic and fish parameters between different sampling periods in summer (S) and winter (W) of 2007, 2008 and 2009. Arithmetic means and *F*-values are provided, except for comparisons that did not meet the assumptions of the analysis of variance, for which medians and the Kruskal-Wallis statistic (*H*) are given. Asterisks indicate significant differences: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . GSI: gonadosomatic index; (-): no data available

	S07	W07	S08	W09	Statistic
Surface temperature (°C)	26.45 <sup>a</sup>	14.11 <sup>b</sup>	26.86 <sup>a</sup>	11.16 <sup>c</sup>	$F = 15812.13^{***}$
Surface salinity	34.37 <sup>c</sup>	36.57 <sup>a</sup>	34.29 <sup>d</sup>	36.40 <sup>b</sup>	$H = 18.42^{***}$
Mean temperature in the water column (°C)	17.86 <sup>a,b</sup>	14.72 <sup>c</sup>	18.90 <sup>a</sup>	11.36 <sup>d</sup>	$H = 90.00^{***}$
Mean salinity in the water column	38.02	37.18	37.85	36.74	$H = 5.30$
Mesozooplankton biomass (mg m <sup>-3</sup> )	29.02 <sup>b</sup>	36.40 <sup>a</sup>	24.70 <sup>b</sup>	28.05 <sup>b</sup>	$F = 7.54^{***}$
Microplankton biomass (mg m <sup>-3</sup> )	32.18 <sup>c</sup>	91.04 <sup>b</sup>	22.70 <sup>c</sup>	130.73 <sup>a</sup>	$F = 76.43^{***}$
Mean chl a in the water column (µg l <sup>-1</sup> )	0.14 <sup>c</sup>	1.36 <sup>a</sup>	0.13 <sup>c</sup>	0.76 <sup>b</sup>	$F = 98.89^{***}$
Catch per unit effort (kg h <sup>-1</sup> )	47.45 <sup>a</sup>	26.45 <sup>a</sup>	5.27 <sup>b</sup>	7.68 <sup>a,b</sup>	$F = 3.40^*$
Condition of adults	1.07 <sup>b</sup>	1.03 <sup>c</sup>	1.09 <sup>a</sup>	1.02 <sup>d</sup>	$F = 4517.40^{***}$
GSI	0.21 <sup>c</sup>	3.91 <sup>b</sup>	0.23 <sup>c</sup>	4.30 <sup>a</sup>	$H = 338.30^{***}$
Condition of juveniles	0.62	–	0.61	–	$F = 1.49$

<sup>a>b>c>d</sup> Homogenous groups

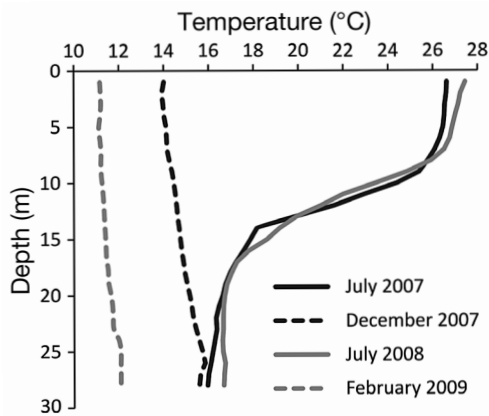


Fig. 4. Averaged profiles of water temperature in the sampling area

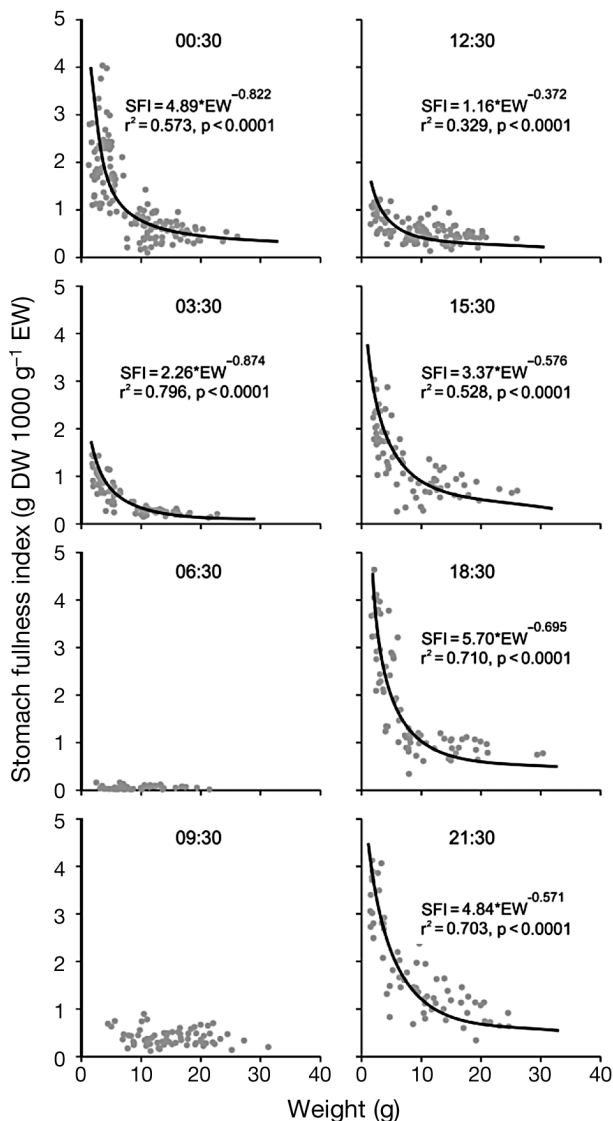


Fig. 5. *Sardina pilchardus*. Stomach fullness index (SFI) against eviscerated weight (EW) for each 3 h time interval in July 2007 (mean time is given, i.e. 00:30 represents 23:00 to 2:00 h). Significant power relationships are also given

tween  $R$  and surface (5 m) temperature ( $T$ ) ( $R = 0.075 \times e^{0.038T}$ ,  $n = 6$ ,  $r^2 = 0.785$ ,  $p < 0.05$ ).

Estimates of consumption by the models of Elliot-Persson and Eggers (Table 3) were highly correlated ( $r = 0.999$ ,  $p < 0.001$ ). Daily ration of juveniles in terms of percent TW was higher (4.25 and 5.36% for S07 and S08, respectively, based on  $C_{E-P}$ ) than that of adults (2.05 and 3.14% for S07 and S08, respectively, based on  $C_{E-P}$ ) in summer (Table 3), presumably because of their smaller size. In adults, daily ration was higher during S08 (3.14%) and W09 (3.67%), with lower consumption estimated in S07 (Table 3). Finally, consumption in W07 was higher than in S07. Higher consumption in S08 and W09 was coupled with lower sardine CPUEs (Table 1). When the ratio of mesozooplankton biomass to sardine CPUE [i.e.  $B_R = \log(\text{mesozooplankton biomass}/\text{CPUE})$ ] was related to daily ration, a significant relationship emerged: daily ration ( $C_{E-P}$ , %TW) estimated was significantly and linearly related to  $B_R$  when the size-class factor (juveniles or adults) was taken into account (Table 4, Fig. 7A). Indeed, mean weight of juveniles and adults (juveniles: 4.82 g TW, adults: 12.81 g TW) entered into the  $C_{E-P}$ -on- $B_R$  regression as a significant covariate (Fig. 7B), indicating that, if a linear decrease in food consumption with fish size is assumed, consumption in terms of %TW decreases by a factor of 0.255  $g^{-1}$  increase in TW.

## DISCUSSION

In contrast to upwelling areas, the Mediterranean Sea is highly oligotrophic and has often been characterized as a marine desert (Azov 1991). However, certain coastal areas under the influence of low-salinity waters are relatively productive and contain important habitats for small pelagic fish stocks in the Mediterranean (Palomera et al. 2007). In the present study, the sampling area (in the coastal area in the proximity of the Nestos River) is a 'hot-spot' for small pelagic fish stocks in the Aegean, with high concentrations of both adults and juveniles (Tsagarakis et al. 2010). In summer, sardine *Sardina pilchardus* populations are dominated by juvenile fish (Machias et al. 2008) that are mainly distributed in shallow waters, especially in the proximity of river mouths (Palomera et al. 2007, Tsagarakis et al. 2010).

The abrupt change in sardine allometric growth, indicated by the significant change in slope of the weight-length relationship at about 100 mm TL, seemed to be related to the ontogenetic transition from juvenile to adult. Indeed, during the winter sam-

Table 2. *Sardina pilchardus*. Analysis of deviance table for the gamma-based generalized linear model fitted to sardine stomach content dry weight. df: degrees of freedom; Time: sampling time; EW: eviscerated weight; \*\*\*p < 0.0001

Source of variation	df	Deviance	Explained deviance (%)	Residual df	Residual deviance	p-value
<b>Juveniles</b>						
Null				516	374.16	
Cruise	1	13.63	3.65	515	360.52	***
Time	7	288.22	77.03	508	72.31	***
EW	1	16.06	4.29	507	56.24	***
Cruise × Time	7	20.02	5.35	500	36.22	***
Time × EW	7	3.00	0.80	493	33.22	***
Total			91.12			
<b>Adults</b>						
Null				1416	1149.76	
Cruise	3	105.07	9.14	1413	1044.68	***
Time	7	486.16	42.28	1406	558.52	***
EW	1	59.68	5.19	1405	498.85	***
Cruise × Time	21	206.98	18.00	1384	291.87	***
Cruise × EW	3	5.02	0.44	1381	286.85	***
Total			75.05			

pling periods, all fish >100 mm were sexually mature, as indicated by the high GSIs (Table 1) and macroscopic staging of gonads. Furthermore, juvenile schools in summer differed in shape and vertical positioning when compared to adult schools (Anonymous 2010); the latter were mainly dense formations positioned near the sea bottom, whereas juvenile schools were ‘ribbon-shaped’ and distributed in mid-waters. The mean length at change from ribbon-shaped to regular sardine schools has been estimated at 105 mm (Anonymous 2010), which corresponds closely to the estimated change in the slope of the weight–length relationship. These observations justified the classification of sardine samples into adults

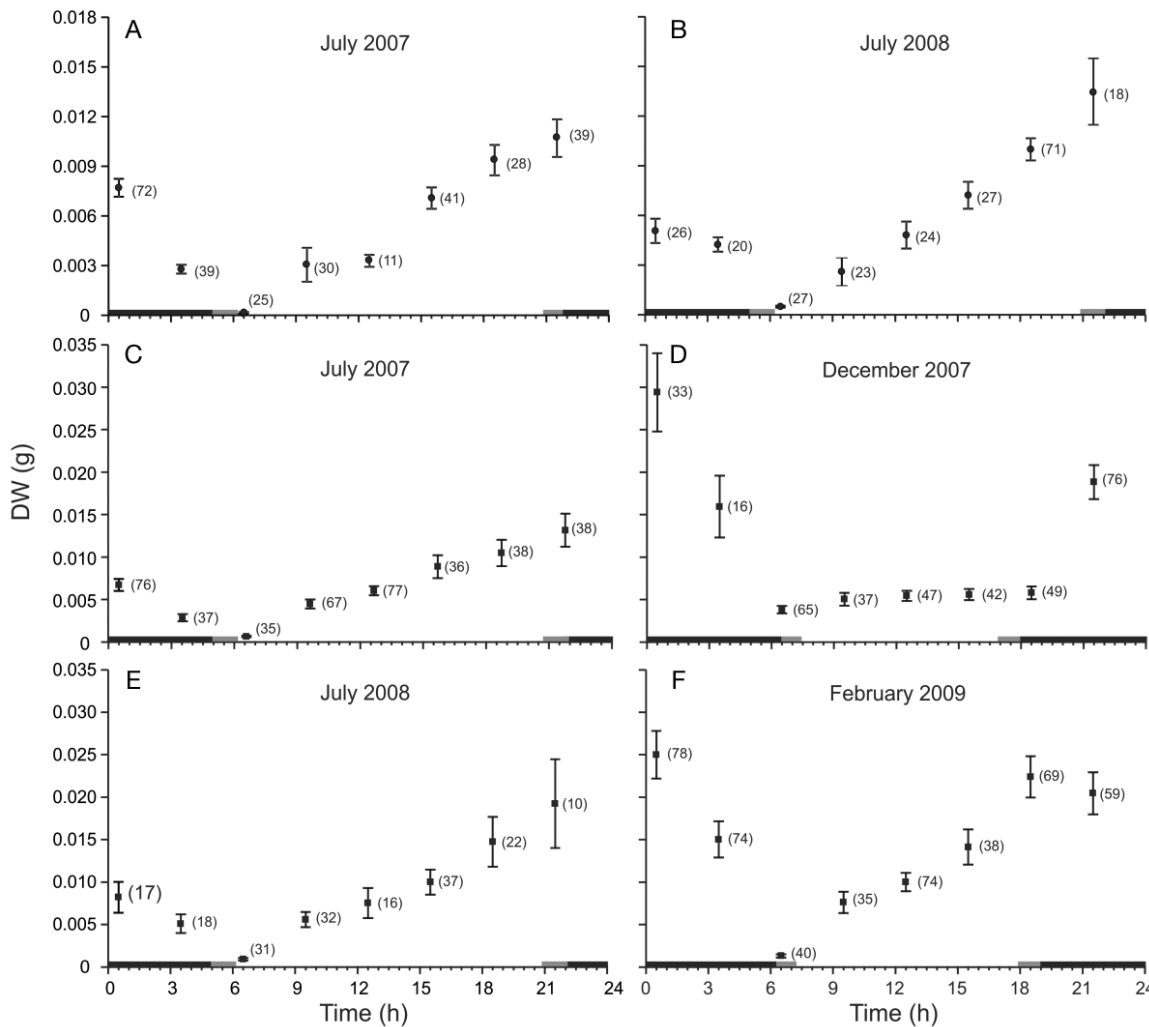


Fig. 6. *Sardina pilchardus*. Estimated marginal means of stomach content dry weight (DW, g), with 95% confidence intervals for juvenile (A & B) and (C to F) adult fish. The numbers of individuals analyzed are given in parentheses. Nautical twilight (grey bar) and nighttime (black bar) are also shown on the bottom axes



Table 3. *Sardina pilchardus*. Estimates of gastric evacuation ( $\text{h}^{-1}$ ) and consumption rates ( $\text{DW d}^{-1}$ ) for sardine. Consumption values are also expressed as percent total weight (%TW). S: summer; W: winter; A: adult sardines; J: juvenile sardines;  $R_{\text{max}}$ : gastric evacuation rate; DW: dry weight, standardized mean stomach content;  $C_{\text{E-P}}$ : daily ration according to the Elliott-Persson model;  $C_{\text{E}}$ : daily ration according to the Eggers model

Sampling period	Size class	$R_{\text{max}}$ ( $\pm$ SE)	DW	$C_{\text{E-P}}$ ( $\pm$ SE)	$C_{\text{E-P}}$ (%TW)	$C_{\text{E}}$	$C_{\text{E}}$ (%TW)
S07	J	0.197 (0.053)	0.0054	0.0266 (0.0029)	4.25	0.0262	4.18
S08	J	0.215 (0.099)	0.0065	0.0335 (0.0053)	5.36	0.0332	5.31
S07	A	0.196 (0.042)	0.0071	0.0342 (0.0031)	2.05	0.0337	2.02
W07	A	0.101 (0.038)	0.0181	0.0426 (0.0015)	2.56	0.0443	2.66
S08	A	0.225 (0.026)	0.0098	0.0524 (0.0053)	3.14	0.0520	3.12
W09	A	0.139 (0.060)	0.0145	0.0611 (0.0532)	3.67	0.0603	3.62

Table 4. *Sardina pilchardus*. Analysis of variance table for the ANCOVA model fitted to sardine consumption ( $C_{\text{E-P}}$ , as percent total weight).  $B_{\text{R}}$ :  $\log(\text{mesozooplankton biomass/Catch per unit effort, CPUE})$ . Adj.  $r^2 = 0.936$

Source	Sum of squares	df	Mean square	$F$	p-value
Model	6.892	2	3.446	37.647	0.008
Intercept	45.372	1	45.372	495.689	0.000
$B_{\text{R}}$	1.822	1	1.822	19.905	0.021
Size class	5.509	1	5.509	60.189	0.004
Error	0.275	3	0.092		
Total	80.877	6			
Corrected total	7.167	5			

( $\geq 100$  mm) and juveniles ( $< 100$  mm) in order to examine feeding periodicity and consumption. In the Pacific sardine in South Africa, smaller sardines (Age 0+) had larger daily rations than larger fish (Ages 1+, 2+, 3+, 4+) (van der Lingen 1998), implying that juvenile fish might have higher energy requirements.

Juvenile sardines in the Mediterranean invest primarily in growth during summer, with no visible mesenteric fat (Machias & Tsimenides 1995, authors' pers. obs.) in contrast to adult sardines which store lipids during summer, mainly to fuel egg production in winter (Ganias et al. 2007, Garrido et al. 2008). Adult sardines in the present study were in better somatic condition in summer, which agrees with the known cycles of energy storage and reproduction in this species (Ganias et al. 2007).

The SFI is widely applied in studies of food consumption in fishes; however, it should be used with caution. As with other ratio indices (e.g. the GSI [Somarakis et al. 2004a] and condition index [Bolger & Connolly 1989]), the SFI is valid for comparisons among samples only when the assumption of isometry between stomach content weight and body weight is met (H eroux & Magnan 1996, Somarakis et al. 2004a). In the present study, a strong dependency of the SFI on fish weight was identified which invalidated its use. A GLM approach was therefore fol-

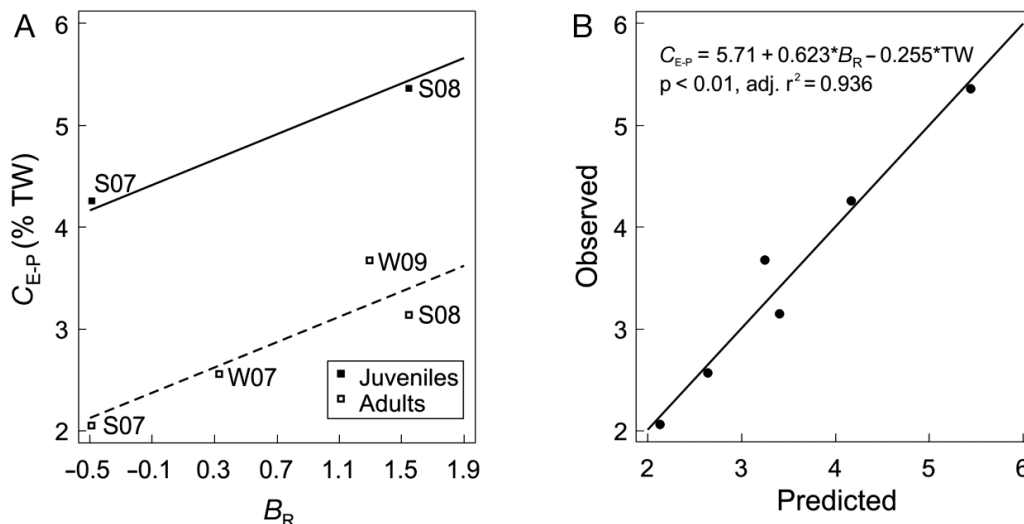


Fig. 7. *Sardina pilchardus*. (A) ANCOVA model of consumption ( $C_{\text{E-P}}$ ) versus mesozooplankton biomass/catch per unit effort ( $B_{\text{R}}$ ). (B) Observed versus predicted values of the multiple regression of  $C_{\text{E-P}}$  on  $B_{\text{R}}$  and fish total weight (TW)

lowed instead to standardize stomach content DWs.

In summer, both juveniles and adults fed during daytime, with a clear peak in feeding intensity at around sunset. In winter, the highest stomach contents were recorded after sunset, in the early night. Andreu (1969), based on opportunistic samples collected throughout the Mediterranean, suggested that sardine feeds during daytime. In the Adriatic Sea, the greatest feeding intensity was noted at dusk during the spring-to-autumn fishing season (Vučetić 1955). Gómez-Larrañeta (1960), however, claimed that sardine feeding goes on throughout the night.

In the present study, sardines exhibited high stomach fullness at dusk and in the early night when luminance levels were low. Feeding in low-light regimes is a general characteristic of clupeoid fish (Blaxter & Hunter 1982). Sardines can feed effectively at luminance levels  $<0.2$  mc (Muzinic 1964) and are able to use filter or particulate feeding over a broad prey size spectrum very efficiently (Garrido et al. 2007), shifting this feeding strategy according to prey concentration (van der Lingen 1994). Thus, a preliminary analysis of prey types in the stomachs of specimens in the sampling cruises of the present study (authors' unpubl. data) revealed that phytoplankton contributed numerically ~79% to sardine diet in winter, which suggested that filtering was probably the feeding mode employed at night. Some feeding activity was also recorded during the day, and this behavior has been observed for other small pelagic fish that are organized into schools (Fréon & Misund 1999 and references therein). Schooling behavior results in enhanced vigilance and reduced timidity, enabling individuals to allocate more time to feeding, even in the presence of predators (Fréon & Misund 1999 and references therein).

The high intensity of feeding of sardines during their ascent to surface waters could be linked with diel vertical migration (DVM) behavior. There are several factors that potentially affect (e.g. light, food, tides), and hypotheses that attempt to explain, the DVM behavior of fish (e.g. bioenergetic advantage, predation avoidance, optimization of different functions) (Neilson & Perry 2001). The visual stimulus required for the maintenance of schools (Blaxter & Hunter 1982, Fréon & Misund 1999, Bertrand et al. 2008) is reduced in the period before sunset when light is diminishing. This leads to the relaxation of sardine school formations and the parallel initiation of the DVM that takes place approximately 1 h before sunset in the Mediterranean (Giannoulaki et al. 1999). Sardine stomachs were full (summer) or almost full (winter) around sunset, suggesting that

the ascent towards surface waters and elevated feeding rates are probably synchronized. Maximization of food intake during the loosening of dense schools and initiation of DVM have also been reported for other small pelagic fish, e.g. Peruvian anchoveta *Engraulis ringens* (Bertrand et al. 2008). Mortality risks might also be reduced at low-luminance levels, as predators of small pelagic fish attack schools mainly during daytime (e.g. marine mammals, birds, fish predators) and based primarily on visual stimuli (Fréon & Misund 1999). Thus, the reduced luminance around sunset triggers the loosening of schools, while concurrently reducing predation risk. There is therefore an increased scope for food intake during dusk and at night.

One possible explanation for the different feeding patterns of sardines in summer and winter is the difference in thermal stratification. The high temperature of surface waters in summer relative to subsurface layers (Fig. 4) could explain the termination of feeding after sardines have ascended close to the surface at night. Food intake by fish increases with increasing temperature, reaches a peak and then falls more or less dramatically at supra-optimal temperature (Jobling 1998). In sockeye salmon *Oncorhynchus nerka*, for example, a significant reduction in appetite and conversion efficiencies was noted at water temperatures from 20 to 24°C (Brett & Higgs 1970), possibly reflecting limitations in the capacity of the respiratory and circulatory systems to deliver oxygen to tissues under conditions of very high oxygen demand (Jobling 1997); such conditions are very likely to be met during searching, capturing and handling of prey. The high temperatures experienced by sardine above the thermocline in summer might therefore limit food intake.

Measurements of gastric evacuation rates in sardines and anchovies under controlled laboratory conditions have rarely been carried out, mainly due to difficulties in keeping small-sized clupeoid fish in experimental tanks (Blaxter & Hunter 1982). In our study, the  $R$  used for the calculation of daily ration was the highest evacuation rate ( $R_{\max}$ ) observed for all pairs of consecutive hauls performed during the same day (for similar approaches see, e.g. Boisclair & Leggett [1985] and Héroux & Magnan [1996]). The estimation of gastric evacuation rates in the field requires making a number of assumptions (e.g. no feeding takes place during the designated non-feeding period, captured fish have the same feeding history, temperature conditions do not change over the 24 h cycle) (Bernreuther et al. 2008). Additionally, the gastric evacuation rate in fish is known to be affected

by a variety of factors, including prey type and size, meal type, predator type and temperature (for a review see Bromley 1994).

In the present study, the increasing degree of digestion of prey items as the night progressed seemed to justify the definition of non-feeding periods. In summer, sardines experienced varying temperatures over the diel cycle owing to their DVM behavior and the thermal stratification of the water column. Hence, the  $R_{\max}$  values calculated during the non-feeding period at night (when fish were located close to the surface) could be higher than actual evacuation rates in the daytime, when fish were distributed below the thermocline. This, in turn, would lead to an overestimation of the consumption rates in summer. Similar exponential increase in  $R$  with increasing temperature, such as that reported in the present study, has also been found in a number of experimental studies (e.g. Elliott 1972, Bernreuther et al. 2008, 2009). When this relationship was used to recalculate the evacuation rate at the depth occupied by sardines during daytime in summer (i.e. below the thermocline), the resulting mean  $R$  values for S07 and S08 were lower by ~23 and ~34 % for juveniles and adults, respectively. Using the recalculated values of  $R$  in the consumption models, daily ration estimates were lower by ~15 and ~22 % for juveniles and adults, respectively (average of  $C_{E-P}$  in S07 and S08). However, in a number of studies, higher evacuation rates have been observed during the feeding period than in the non-feeding period (Richter et al. 2002 and references therein), but see also Bernreuther et al. (2008) for an exception in *Clupea harengus*. Hence, even if a temperature correction was applied for  $R$  in summer during daytime, it was likely that an increase of  $R$  during the feeding period would counteract the expected decrease in  $R$  due to low temperature below the thermocline. Finally, in sprat *Sprattus sprattus*, the general model describing the  $R$ - $T$  relationship predicts an increase of  $R$  with  $T$ , but a subsequent decrease at higher  $T$  (Bernreuther et al. 2009). If the latter is also the case for sardine above the thermocline, the calculated  $R_{\max}$  during nighttime could be a realistic value to implement in the consumption models.

Regarding other factors affecting  $R$ , the effect of fish size is debated, as in some studies no significant effect was noted (e.g. Persson 1979), whereas, in others, the effect of size was significant (e.g. Temming & Herrmann 2001, Bernreuther et al. 2009). Prey type is also known to affect  $R$  in Pacific sardines, with phytoplankton prey leading to higher  $R$  values than zooplankton (van der Lingen 1998). If such an effect

holds true for Mediterranean sardine as well, it could partially explain the higher  $R_{\max}$  values in W09 ( $0.139 \text{ h}^{-1}$ ) than in W07 ( $0.101 \text{ h}^{-1}$ ). Although temperature was significantly lower in W09 and  $R_{\max}$  was expected to be lower than that of W07, the numerical contribution of phytoplankton in sardine diet was ~83 % in W09, while the respective proportion during W07 was ~73 % (authors' unpubl. data).

Bearing in mind the above difficulties in estimating  $R$  from field data, the estimated values of the gastric evacuation rate obtained with the  $R_{\max}$  approach (Boisclair & Leggett 1985, Héroux & Magnan 1996) were considered the most appropriate for the present study. Gastric evacuation rates obtained for other clupeoids like Atlantic herring *Clupea harengus*, Pacific sardine *Sardinops sagax* and Baltic sprat *Sprattus sprattus* range from  $0.09$  to  $0.56 \text{ h}^{-1}$  (Arrhenius & Hansson 1994, van der Lingen 1998, Darbyson et al. 2003, Bernreuther et al. 2008, 2009). However, direct comparisons of gastric evacuation between species are probably meaningless due to the dependency of evacuation rate on temperature and food type (van der Lingen 1998) and whether  $R$  is calculated using DW or SFI values (Héroux & Magnan 1996).

Two widely used consumption models proposed by Elliott & Persson (1978) and Eggers (1979) were applied here for comparative purposes. The E-P model is generally considered to be accurate (Hayward 1991) and to perform better in filter feeders and grazers that demonstrate feeding periodicity (Richter et al. 2002). The Eggers model, on the other hand, is considered more appropriate for species that feed throughout the day on a wide range of prey types, exhibiting occasional feeding peaks and having no rigid feeding periodicities (Héroux & Magnan 1996). In the case of sardine, the 2 models produced very similar consumption estimates, as was also the case in other studies (Boisclair & Leggett 1988, Hayward 1991, Héroux & Magnan 1996, Richter et al. 2002). Although the key assumption of the Eggers model, i.e. continuous feeding, was not met in the present study, it provided very close estimates to the E-P model, suggesting that it could be used instead. The Eggers model is simpler but still robust, permitting lower sampling frequency than the E-P model (Boisclair & Leggett 1988, Héroux & Magnan 1996). However, it may overestimate daily ration, particularly at longer sampling intervals and at medium- and high-ration levels (Hayward 1991).

The estimated daily ration values for sardine exhibited seasonal and inter-annual variability. The feeding intensity of sardine has also been reported to vary between years and seasons, being higher when

productivity is high, especially during the spring and winter months off the Atlantic coasts of Portugal (Garrido et al. 2008). In our case, summer consumption values were lower than those of the following winter, when chl *a* and plankton biomass were both higher. Despite significant differences in mean zooplankton biomass and chl *a* concentration among seasons, consumption rates were not significantly correlated with either zooplankton or chl *a*. Daily ration is known to vary with type of food because of differences in gastric evacuation rates for different prey types (van der Lingen 1998). In Pacific sardine *Sardinops sagax*, field estimates of daily ration ranged from 0.99 to 2.52 and from 2.97 to 7.58% TW d<sup>-1</sup> for fish consuming zooplankton and phytoplankton, respectively (van der Lingen 1998). The latter author provided additional estimates of 1.73 to 5.18 and 2.31 to 6.93% TW for far eastern and Peruvian sardines, respectively. In laboratory experiments, the daily ration of 1 yr old Pacific sardines fed synthetic pellets ranged from 1.06 to 2.40% TW (Noguchi et al. 1990). Finally, from back-calculated growth data, Watanabe & Saito (1998) estimated that the daily requirements in carbon of juvenile sardines ranged from 5.6 to 9.6 mg C d<sup>-1</sup>, which, according to the conversion factors used in their study, corresponded to daily rations of 3.52 and 5.68% TW for fish of ~0.83 and ~0.30 g TW, respectively.

In the Mediterranean Sea, the only available daily ration estimates for small pelagic fish are those for European anchovy *Engraulis encrasicolus* (Tudela & Palomera 1995, 1997, Plounevez & Champalbert 2000). Using an evacuation rate of 0.423 ± 0.196 h<sup>-1</sup> (estimated in Tudela & Palomera 1995), adults of this species have been found to consume from 3.4 to 3.9% TW during the summer spawning period. Furthermore, anchovy studies in the Mediterranean (Tudela & Palomera 1995, 1997, Plounevez & Champalbert 2000, Borme et al. 2009) indicate a diel feeding intensity pattern similar to that of sardine in the present study during summer, i.e. daytime feeding, with peak intensity at around sunset.

One of the most interesting findings of the present study was the positive relationship between consumption and the index of per capita food availability, i.e. the biomass ratio, log(mesozooplankton biomass/CPUE) (Fig. 7). This relationship is indicative of a density-dependent effect, i.e. when per capita food availability is increased, daily ration is higher.

Density-dependence responses have increasingly been identified in small pelagic fish including expansion–contraction of habitats (e.g. Somarakis et al. 2004b) and changes in somatic condition, gonad

mass and length at maturity (van der Lingen et al. 2006). In the Aegean Sea, the daily specific fecundity of anchovy is strongly and positively related to the ratio of mesozooplankton biomass to fish biomass (Somarakis et al. 2011). This is to our knowledge the first time that a relationship between per capita food availability and consumption has been demonstrated. The implications are important, especially in coupled biogeochemical and fish bioenergetics models in which small pelagic fish consumption is modeled as a function of prey density (e.g. Politikos et al. 2011) without taking into consideration any density-dependent control.

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