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Temporal variation in prey selection by adult European sardine (*Sardina pilchardus*) in the NW Mediterranean Sea

Chia-Ting Chen^{*}, François Carlotti, Mireille Harmelin-Vivien, Loïc Guilloux, Daniela Bănaru

Aix-Marseille Université et Université de Toulon, Mediterranean Institute of Oceanography (MIO), CNRS/INSU, IRD, UM 110, Campus universitaire de Luminy, case 901, 13288 Marseille cedex 9, France

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ABSTRACT

In the North-Western Mediterranean Sea (NWMS), changes in size and taxonomic composition of zooplankton have been hypothesized to underlie observed changes in populations of *Sardina pilchardus*, one of the main small pelagic fish species. The present study was undertaken to better understand the link between the temporal variability of zooplankton and the diet of adult sardine in the Bay of Marseille over a period of 16 months by comparing the taxonomic and prey size composition of sardine stomach content with net-collected zooplankton samples from a fixed station. A greater similarity was found between sardine stomach content and 80- μm mesh size net samples than with the traditionally used 200- μm net samples. Sardine showed a high diet plasticity over the entire study period, covering a broad diversity of prey species and a wide prey size range (ranging from 400 to 1000 μm in measured total length), including a lot of small copepods (*Microsetella/Macrosetella* spp., Corycaeidae and Oncaeidae). The most consumed prey groups were not necessarily the most abundant in the zooplankton sampled with nets, suggesting potential prey selection. Sardine showed most often an opportunistic selective predation on larger individuals for most taxonomic prey groups. Results highlighted the diet flexibility of sardine between months and showed seasonal variation in prey size, suggesting a continuous filter-feeding mode on relatively small prey when prey densities were high and more frequent selective particulate-feeding on larger prey when prey densities were low. Sardines were able to feed on a wide diversity of prey and size range and thus to shift their diet between months. This ability probably allows them to adapt to high spatial and temporal variability of the zooplankton community in a context of both human-induced and climatic changes.

1. Introduction

The European sardine, *Sardina pilchardus* (Walbaum, 1792), is one of the most important small pelagic species in terms of biomass, abundance and catches in the Mediterranean Sea (Palomera et al. 2007), representing 39% of landings in the Gulf of Lion (Bănaru et al. 2013) and about 23% of the annual landings in the Mediterranean Sea (FAO 2020). Bottom-up changes in the pelagic food web induced by climate change through hydrological processes are suspected to be one of the major drivers of sardine populations in many ecosystems, including the North-Western Mediterranean Sea (NWMS) (Moullec et al. 2016 and references therein). These changes at the base of the food web with regard to sardine stocks of the NWMS are highlighted by strong correlations between stocks and hydrological parameters at decadal scales (Loret et al. 2004; Palomera et al. 2007) or with climate indices (Martín et al. 2012).

More recent studies documented changes in sardine relative body condition in the Gulf of Lion and the Catalan coast (below the NWMS shelf) in relation with environmental drivers (STECF 2015; GFCM 2019) and highlighted the potential major role of the mesozooplankton as the missing link between the environmental drivers and sardines (Van Beveren et al. 2014; Quattrocchi and Maynou 2017; Coll et al. 2018; Saraux et al. 2019; Feuilloley et al. 2020). Therefore, two major approaches should improve our understanding of zooplankton-sardine interactions in the NWMS: to better document the zooplankton prey availability and diversity in the fish habitats, and to better understand the feeding habits of sardines (Saraux et al. 2019).

In the NWMS, the observed shifts in sardine stocks were due to a decline in their body condition and not to lower recruitment (Saraux et al. 2019), and are therefore suspected to be more linked to feeding conditions of adults rather than to those of larvae and juveniles.

^{*} Corresponding author.

E-mail addresses: chiating.chen9692@gmail.com (C.-T. Chen), francois.carlotti@mio.osupytheas.fr (F. Carlotti), mireille.harmelin@mio.osupytheas.fr (M. Harmelin-Vivien), loic.guilloux@mio.osupytheas.fr (L. Guilloux), daniela.banaru@mio.osupytheas.fr (D. Bănaru).

However, both the trophic dynamics of sardine and the zooplankton prey dynamics on the NWMS shelf still need to be better documented to fully describe the feeding ecology of the sardine population and its potential plasticity and flexibility in response to environmental changes (van der Lingen et al. 2009; Saiz et al. 2014; Moullec et al. 2016; Saraux et al. 2019).

For the NWMS shelf, most studies on sardine feeding ecology are mainly based on stomach content analysis of individuals caught during the summer stock assessment surveys (Costalago et al. 2012; 2014; Costalago and Palomera 2014; Le Bourg et al. 2015; Bachiller et al. 2020), except for a few cases of seasonal observations (Costalago et al. 2012; 2014; Costalago and Palomera 2014). Sardine diet analyses performed in the Gulf of Lion in different years over the last decade have highlighted major changes compared to previous studies (Costalago and Palomera 2014; Le Bourg et al. 2015). Bachiller et al. (2020) indicated opportunistic ingestion of available prey by sardine and observed a latitudinal gradient of variation in the sardine trophic ecology. This observed gradient along the NWMS coast coincides with previously described poorer feeding conditions for the northern Mediterranean populations with smaller prey compared to those of the southern populations. Feeding conditions in terms of water temperature (influencing prey taxonomic and size composition) and turbidity (influencing visual capacity) may also influence the diet of planktivorous species (Salonen et al. 2009; Higham et al. 2015).

However, to date, there has been no detailed study on the trophic ecology of adult sardine in the Gulf of Lion in relation to the monthly variation of its prey dynamics, which could help to better characterize the diet of the NW Mediterranean sardines.

Sardines feed either by non-selective filtration (sieving plankton from the water with gill rakers) or by particulate-feeding (active selective pursuit and capture of individual prey items) or by both modes (van der Lingen 1994; van der Lingen et al. 2009). Therefore, sardine are able to feed on a wide size-spectrum of plankton prey, but they derive most of their dietary energy from zooplankton (Bode et al. 2004; Garrido et al. 2008; van der Lingen et al. 2009; Costalago et al. 2012; Costalago and Palomera 2014) even if their phytoplankton consumption by filtering

might be occasionally substantial (van der Lingen 1994) and sometimes toxic (van der Lingen et al. 2016). It is thus essential to consider the influence of seasonal changes of feeding conditions, based on community structure and size spectra of zooplankton, on sardine prey selectivity for a better understanding of the mechanisms linking zooplankton and sardine diet.

In order to provide more comprehensive information on the functional ecology of sardine feeding, the hypotheses to be tested in the present study are the following: i) prey selection of sardine in terms of taxonomic groups and sizes may vary temporally, and ii) both trophic plasticity and flexibility enable sardines to adapt their feeding to the temporal variation of zooplankton communities. Therefore, the present study consists in a precise analysis of the taxonomic composition and size of zooplankton prey found in sardine stomachs compared to the zooplankton community available in the environment over a period of 16 months in the Bay of Marseille.

2. Material and methods

2.1. Study area

Located in the eastern part of the Gulf of Lion, the Bay of Marseille (South of France, NWMS) is subject to various hydrological fluctuations (Fig. 1): southwards by intrusions of the offshore Liguro-Provençal current (also called Northern Current; Petrenko et al. 2005), occasionally westwards by intrusions of the Rhone river plume, and by the coastal Huveaune River which flows into the center of the bay and which is subject to strong flood inputs depending on wind and rain events (Cresson et al. 2012; Fraysse et al. 2014). This bay is also subject to anthropogenic and terrestrial inputs from the Marseille sewage treatment plant at Cortiou in the southeastern part of the bay (Millet et al. 2018). Thus, three main potential sources of particulate organic matter with different biochemical characteristics - marine particles, river runoff and sewage water particles - are available to zooplankton (Bănaru et al. 2014; Chen et al. 2019). Mesozooplankton communities of the Bay of Marseille have been shown to be representative of those of the Gulf of

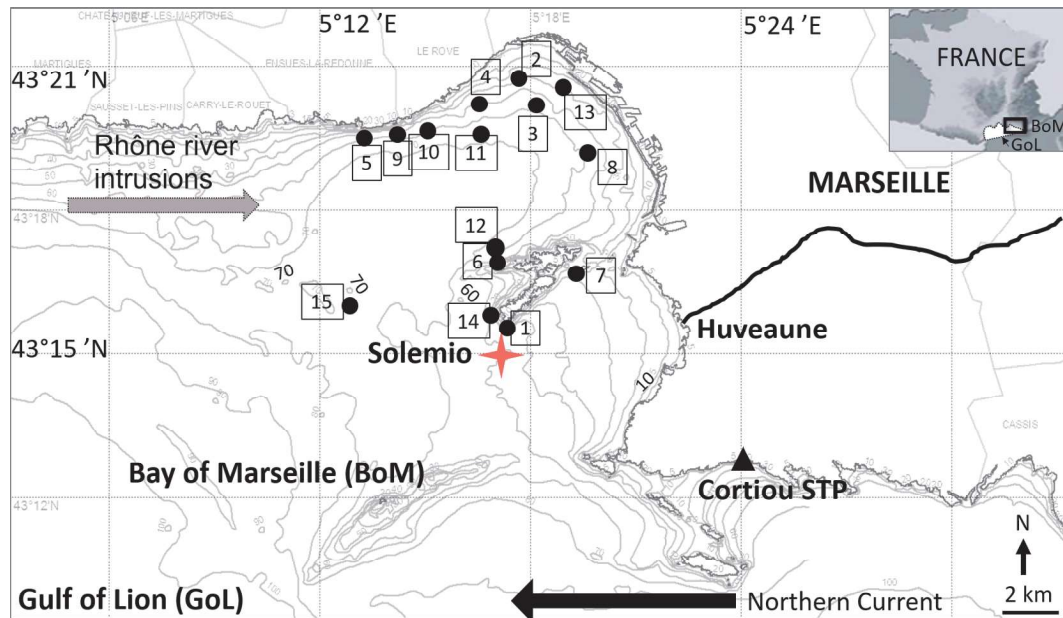


Fig. 1. Fishing sites of *S. pilchardus* (black points) with framed numbers corresponding to the month in Table 1 and Solemio sampling site of zooplankton (43.24°N; 5.29°E) (red diamond point), in the Bay of Marseille (BoM), located in the eastern part of the Gulf of Lion (GoL), NW Mediterranean Sea. The Solemio site may occasionally be influenced by intrusions of the Rhone river plume (grey arrow) and the coastal Huveaune River (bold line), depending on wind and rain events. This site may also be influenced by intrusions in the bay of the offshore Northern Current (black arrow), and subject to anthropogenic and terrestrial inputs from the Marseille sewage treatment plant at Cortiou (Cortiou STP, black triangle). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Lion (Espinasse et al. 2014).

2.2. Sample collection

Sardines and zooplankton were sampled monthly in the Bay of Marseille from December 2016 to March 2018. Once or twice a month, sardine samples were bought early in the morning from the local fishermen working with lampara and gillnets within the Bay of Marseille (Fig. 1, Table 1). In November 2017, sardine samples were not obtained due to continuous bad weather conditions. Once caught, sardine samples were immediately put on ice aboard to stop prey digestion and kept at -20°C after arrival at the laboratory.

Zooplankton sampling was performed at the Solemio site (43.24°N ; 5.29°E) (Fig. 1), on board the RV ANTEDON II. The observation program at the Solemio site contributes to the long-term national program for littoral observation, SOMLIT (<http://somlit.epoc.u-bordeaux1.fr>), and includes a wide range of physical and chemical environmental parameters that have been recorded bi-monthly for several decades (monthly mean values of selected environmental parameters used in the present study are available in the Supplementary Materials). In the frame of the SOMLIT monitoring program, zooplankton is sampled vertically, from 55 m depth (close to the bottom) to the surface, with a WP2 200- μm mesh size net (50 cm mouth diameter) towed at 1 m s^{-1} to obtain taxonomy and abundance data over the entire study period. The water volume sampled was estimated using depth and net surface.

A preliminary step of this study was to see if this sampling method allows us to catch a size range of zooplankton similar to the prey size range of sardines in the study area. In a test-phase of the present project, we observed that zooplankton prey in sardine stomachs were generally smaller and different from those sampled with the 200- μm mesh size net. Consequently, our sampling was completed with an 80- μm mesh size net (50 cm mouth diameter) towed at 1 m s^{-1} in order to achieve more consistency when comparing sardine prey and net-sampled zooplankton.

Zooplankton and sardine sampling were performed separately in time (one to several days) and space (1 km to 15 km). The zooplankton sampling dates were scheduled at the beginning of each semester in the frame of the SOMLIT program, whereas sardine samples were available depending on fishing dates of the fishermen. Table 1 allows to identify the nearest dates when sardines were sampled before or after zooplankton samplings. In this sense, we consider that the zooplankton sampled at the SOMLIT site is representative of the sardine prey field in

the Bay of Marseille and we expect the long sampling period (16 months) to highlight the diversity of feeding conditions for sardines in this area.

2.3. Sample analyses

Total fish length (TL) was measured to the nearest 0.1 cm and fish weight recorded to the nearest 0.1 g. Fish were then dissected, sexed by the examination of their gonads, and stomachs were kept again in a freezer at -20°C before analysis. Once a stomach had been opened under a binocular microscope, the degree of stomach repletion was estimated visually using a semi-quantitative scale of the surface occupied by prey items when spread out on a Petri dish: 0 = empty, 1 = $> 0 - 25\%$ full, 2 = 25–50% full, 3 = 50–75% full, 4 = 75–100% full. The empty stomachs were discarded prior to further analyses. The Dry Weight (DW) of each organism was obtained from length-weight relationships gathered from the literature (Table S1). The Stomach Fullness Degree (SFD) was used to assess the feeding intensity as done by Bachiller et al. (2016). It is defined as the sum of prey dry weight in the stomach divided by the total length of the fish and is expressed in $\mu\text{g cm}^{-1}$ in Table 1. Due to sampling limitations on phytoplankton, only zooplankton prey were identified and sorted for further taxonomic and size analyses.

The main taxonomic groups of zooplankton found in both sardine stomachs and plankton net samples were identified to the lowest possible taxonomic level under a binocular Leica® M205C microscope using a maximum magnification of 1280x, and their abundance (number of individuals) was recorded. The main copepod groups were identified following the website 'Marine Planktonic Copepods' (<https://copepodes.obs-banyuls.fr/en/loc.php?loc=14>) (Razouls et al. 2005). A total number of 29 prey groups were identified in the sardine diet, among which the 16 most important groups were detailed in Fig. 2a and the remaining 13 groups were grouped in "Others" (Table S1). At least 15 sardines were analyzed per month to reach the maximum number of identified prey groups determined from mean cumulative prey curves (Fig. S1). A total number of 440 sardines, with a mean total length (TL) of $12.80 \pm 1.40\text{ cm}$ and a mean total weight of $15.13 \pm 6.20\text{ g}$, were analyzed.

2.4. Diet characterization

Diet composition was analyzed with several complementary indices: frequency of occurrence (%O, percentage of non-empty stomachs con-

Table 1

Sampling dates of zooplankton and *S. pilchardus* for each month of the study period in the Bay of Marseille. Number of sampled sardine individuals (N), range of total length (TL) and mean length (\pm SD) (cm), number of empty stomachs (ES), mean degree of stomach repletion (SRD) (\pm SD) and stomach fullness degree (SFD) (\pm SD) ($\mu\text{g cm}^{-1}$) of *S. pilchardus* by month. Fish Loc. are the fishing location points of the month with the corresponding number in Fig. 1. Sardines were not sampled in November 2017 because of bad weather conditions. Superscript letters represent post-hoc groups after Kruskal-Wallis tests. Values with similar letters are not significantly different ($P > 0.05$).

Month	Sampling days of the months		Fish Loc.	N	TL (cm)		ES	SRD	SFD ($\mu\text{g cm}^{-1}$)
	Plankton	<i>S. pilchardus</i>			Range	Mean (\pm SD)			
Dec-16	12	6	1	45	11.4–16.8	$13.66 \pm 1.41^{\text{ab}}$	1	2.4 ± 1.2	$4.09 \pm 7.06^{\text{ab}}$
Jan-17	4, 18	19, 20	2	45	10.1–15.6	$12.68 \pm 1.35^{\text{ce}}$	0	2.4 ± 1.4	$10.89 \pm 17.56^{\text{abc}}$
Feb-17	1	22	3	36	10.2–13.6	$11.59 \pm 0.87^{\text{f}}$	2	2.0 ± 1.2	$3.80 \pm 9.13^{\text{ab}}$
Mar-17	1, 14, 29	10, 17	4	45	10.1–15.3	$12.13 \pm 0.87^{\text{cdf}}$	0	2.1 ± 1.2	$4.35 \pm 6.66^{\text{ab}}$
Apr-17	10, 26	6, 8	5	45	12.1–18.1	$14.49 \pm 1.70^{\text{a}}$	0	1.9 ± 1.2	$3.72 \pm 7.19^{\text{a}}$
May-17	10, 24	13, 23	6	44	10.1–16.0	$13.10 \pm 1.88^{\text{abe}}$	1	2.6 ± 1.3	$39.57 \pm 18.73^{\text{d}}$
Jun-17	8, 22	13	7	26	11.6–14.5	$12.91 \pm 1.11^{\text{abcde}}$	0	2.3 ± 1.1	$2.41 \pm 2.75^{\text{abc}}$
Jul-17	4, 19	12	8	23	11.0–13.1	$12.18 \pm 0.72^{\text{cdef}}$	0	2.8 ± 1.2	$7.67 \pm 5.74^{\text{cd}}$
Aug-17	30	17	9	20	11.6–14.1	$12.71 \pm 0.89^{\text{bcde}}$	0	2.0 ± 1.4	$6.72 \pm 10.31^{\text{bcd}}$
Sep-17	6, 18	22	10	23	12.1–14.2	$13.06 \pm 0.78^{\text{abe}}$	7	1.4 ± 1.0	$1.60 \pm 1.75^{\text{ab}}$
Oct-17	11, 18	10	11	19	11.1–15.2	$12.89 \pm 1.36^{\text{bcde}}$	1	2.2 ± 1.4	$1.20 \pm 0.91^{\text{a}}$
Nov-17	2, 16, 29	–	–	–	–	–	–	–	–
Dec-17	13	19	12	20	10.7–13.9	$12.32 \pm 1.09^{\text{cdef}}$	1	1.4 ± 1.0	$1.11 \pm 0.88^{\text{a}}$
Jan-18	24	29	13	15	10.7–13.0	$11.96 \pm 0.66^{\text{cdf}}$	0	3.7 ± 0.9	$23.97 \pm 18.82^{\text{d}}$
Feb-18	21	15	14	16	10.4–13.3	$11.91 \pm 1.08^{\text{cdf}}$	0	1.6 ± 1.0	$2.96 \pm 3.65^{\text{abc}}$
Mar-18	9	16	15	18	10.5–13.8	$11.81 \pm 0.92^{\text{df}}$	0	3.8 ± 0.6	$6.25 \pm 7.81^{\text{abc}}$
Total number of samples	27	19		440					

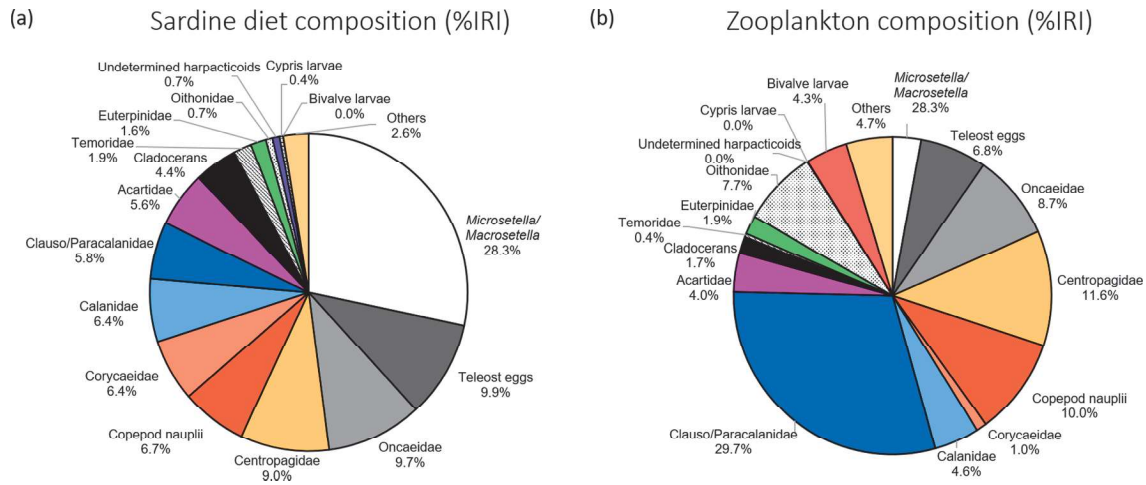


Fig. 2. Average %IRI (index of relative importance) of (a) zooplankton in the diet of *S. pilchardus* and (b) zooplankton sampled with the 80-µm mesh size plankton net over the sampling period from December 2016 to March 2018 in the Bay of Marseille.

taining a given prey item), numerical percentage (%N, mean percentage per stomach of the number of a given prey item related to the total number of all prey in each stomach), and dry weight percentage (%W, mean percentage per stomach of the weight of a given prey item related to the total weight of all prey in each stomach). The index of relative importance (IRI) of taxonomic groups (Pinkas et al. 1971) was calculated according to the formula:

$$IRI = \%O \times (\%N + \%W)$$

The IRI was then transformed to percentage by dividing it by the sum of the IRI for each prey item (%IRI) (Cortés 1997).

In order to compare the community composition of zooplankton sampled with nets with the diet composition of sardine diet, %IRI was also calculated for zooplankton sampled with the 200-µm mesh net during the test-phase of this study, from December 2016 to February 2017, and for zooplankton sampled with the 80-µm mesh net during the entire study period. Monthly %IRI data were analyzed and presented. However, for a better understanding of the seasonality of sardine diet composition, we also analyzed %IRI according to hydrological seasons (winter- January to March, spring- April to June, summer- July to September and autumn- October to December), following Seguin (1981).

Prey composition was also studied following the Costello (1990) graphical representation, with the prey-specific abundance (Amundsen et al. 1996) as a function of the frequency of occurrence. Prey-specific abundance, defined as the percentage a prey taxon comprises of all prey items in only those predators in which the actual prey occurs, was calculated with the formula of Amundsen et al. (1996):

$$P_i = \left(\frac{\sum S_i}{\sum S_i} \right) \times 100$$

where P_i is the prey-specific abundance of prey i , S_i the stomach content (numerical abundance) comprised of prey i , and S_i the total stomach content in only those predators with prey i in their stomach. This graphical method provides a good description of prey importance and is useful to suggest certain diet specialization or main feeding strategy (specialized or generalized) (Bachiller and Irigoien 2015).

We calculated diet diversity per month in order to quantify the diet plasticity. The diet diversity was expressed by the Shannon-Wiener diversity index (H'), calculated from the prey species list (i.e. 17 groups) with the following formula:

$$H' = - \sum_{i=1}^S p_i \times \ln p_i$$

where S , the prey species richness defined as the number of different prey groups found in stomach content and p_i the proportion (%IRI) of the group i .

The maximum length of prey (sum of prosome and urosome lengths for copepods) was measured on at least 30 individuals of representative groups of organisms found in each sardine stomach when possible, and on the first 30 individuals in the zooplankton net sample under a binocular microscope. These size measurements allowed the calculation of prey-predator size ratio (PPSR) between each fish individual and its prey, and the determination of sardine selectivity within a particular prey size range.

A comparison of size spectra of prey organisms from sardine stomach content and of plankton organisms sampled with the 80-µm mesh-size net was performed over the whole study period. An additional comparison with plankton in 200-µm mesh-size net samples was performed as well for a period of 3 months (December 2016 to February 2017). Finally, four copepod groups (*Microsetella/Macrosetella* spp., Oncaeiidae, Corycaeiidae, and Oithonidae), often found in sardine diet in the literature (Costalago and Palomera 2014; Le Bourg et al. 2015), were taken as examples for illustrating the results of monthly variation of prey size.

The trophic niche breadth (TNB) of sardine was examined by determining changes in the range of the Shannon-Wiener diversity index (H') of prey and also PPSR with increasing predator size. The differences between predicted diversity and PPSR values of upper and lower bound regressions at any given predator size represented the TNB (Scharf et al. 2000; Bachiller and Irigoien, 2013; 2015). The diet plasticity of sardine was defined as its capacity to change its diet and to consume a wide diversity of prey taxa and prey size range available in the environment. We used the term 'flexibility' to denote the ability to switch easily between prey types between consecutive months. Prey 'selectivity' and 'preference' are often employed synonymously in the literature (Juanes and Conover 1994). However, prey selectivity (or electivity), often defined as any difference of the relative frequency of a given prey in the stomach content compared to its relative frequency in the environment, can be positive (selected) or negative (avoided) (Graham and Kroutil 2001). While preference usually refers to prey selectivity when all food types are equally available under experimental conditions (Ivlev 1961). Prey items can thus be selectively ingested but not preferred.

To understand the prey selection pattern of sardine and its temporal variations, prey selectivity was estimated with Ivlev's diet selectivity index (E) (Ivlev 1961), calculated with the following equation:

$$E_i = (r_i - p_i) \div (r_i + p_i)$$

where r_i is the proportion of prey item in sardine stomachs, equivalent to

%IRI_i and p_i is the proportion of this prey item i available in the marine environment, equivalent to %IRI_i for the organisms sampled with the 80- μ m mesh net. E_i varies between -1 and 1 . When E is close to 1 , it means that the prey item is selected actively and when it is close to -1 , it means that the prey is avoided actively by the predator.

2.5. Statistical analyses

Data processing was done using the software R (R Core Team 2020). After testing for normality and homogeneity of variances, ANOVA (F) tests or non-parametric Kruskal-Wallis (H) tests were used to examine the significance of monthly differences of several variables: total length of sardines, mean size of zooplankton organisms sampled with the 80- μ m and 200- μ m mesh nets and found in sardine stomach content, the PPSR and the Shannon-Wiener diversity index (H'). Post-hoc comparisons of means were performed with a Dunn's test. ANOSIM (analysis of similarities) was done to test the significance of diet difference between individuals of different sexes. This was done monthly to avoid the effect of month on diet. Spearman's correlation test was used to test the significance of correlation between the monthly mean size of sardine PPSR. The Chi-Squared Test of Independence was used to test whether the differences of taxonomic composition and size distribution between the organisms found in sardine diet and sampled with nets (80- μ m and 200- μ m) were significant. Bray-Curtis dissimilarities distances were calculated accordingly. To illustrate temporal variations, Principal Component Analyses (PCA) were done on monthly data of %IRI of sardine diet and the %IRI composition of zooplankton sampled with the 80- μ m mesh net. This analysis allows to characterize their monthly variations and to highlight which groups explain the most of these variations. Wilcoxon-Mann-Whitney test was used to test if the mean values of Ivlev selectivity were significantly different from zero in order to clarify whether some groups were significantly positively or negatively selected. Bray-Curtis distance was calculated between pair-wise consecutive months to show potential shifts in diet between months so as to quantify trophic flexibility.

3. Results

3.1. Sardine diet composition

The mean total length of sardine varied significantly between months ($H = 98.62$, $P = 0.024$) with larger individuals sampled in April 2017 and smaller individuals sampled in February 2017 (Table 1). The number of sardines with empty stomachs was very low except for September 2017, and the mean stomach repletion degree was 2.3 ± 1.3 (about 50% of stomach fullness). The feeding intensity, expressed by Stomach Fullness Degree (SFD), varied significantly between months ($H = 70.00$, $P < 0.001$), with the highest values in May 2017 and the lowest ones in December 2017 (Table 1). No change of TNB based on prey diversity or PPSR with sardine size (Fig. S2) and no effect of sex (ANOSIM $R = -0.004$, $P = 0.574$) on diet composition was observed, meaning that prey size and diversity did not differ between sardine size classes and sex during the whole sampling period.

Sardine fed mainly on copepods (%IRI = 82.8), with most individuals (58.6%) feeding on *Microsetella/Macrosetella* spp. (Fig. 2a and Fig. S3). Oncaeiidae, Centropagidae, copepod nauplii and Corycaeiidae were also important prey. Teleost eggs, cladocerans, cypris larvae, bivalve larvae and other organisms such as decapod larvae, salps, siphonophores, amphipods, ostracods and pteropods also completed sardine diet but in lower percentages. Sardine showed thus a rather generalist diet, while mainly based on copepods. Copepods also dominated the average composition of zooplankton sampled with the 80- μ m mesh size net (%IRI = 82.5) with Clauso/Paracalanidae as the most important group, followed by Centropagidae, Oncaeiidae and Oithonidae (Fig. 2b). Teleost eggs, bivalve larvae and other organisms such as decapod larvae and amphipods were also observed but in lower percentages.

During the test-phase (December 2016 - February 2017), the composition of the sardine diet was significantly different from those of the 80- μ m and 200- μ m mesh net samples ($\chi^2 = 107.94$, $P < 0.001$ against 80- μ m and $\chi^2 = 142.34$, $P < 0.001$ against 200- μ m). However, a higher similarity existed between the composition of the sardine diet and the 80- μ m mesh net samples than with the 200- μ m mesh net samples (Bray-Curtis distance 0.71 and 0.78, respectively). Sardines consumed high proportions of copepod nauplii and *Microsetella/Macrosetella* spp. copepods, which were present in the 80- μ m mesh net samples, but hardly visible in the 200- μ m mesh net samples (Fig. 3). Inversely, Clauso/Paracalanidae copepods, which dominated in both net samples, were rarely present in sardine stomachs. The mean size of measured prey in sardine stomachs was also significantly different from that of those sampled with both nets ($\chi^2 = 359.61$, $P < 0.001$). The 200- μ m mesh net sampled less frequently small organisms such as cypris larvae, copepod nauplii, *Microsetella/Macrosetella* spp. and Euterpinidae copepods, and captured larger individuals of bivalve larvae, Oncaeiidae, Oithonidae and Calanidae copepods (see Table 2 and the superscript post-hoc letters for $P < 0.05$). The size distribution of prey in sardine diet and plankton sampled with the 80- μ m mesh size did not differ significantly ($\chi^2 = 27.58$, $P = 0.120$) and were more similar between each other (Bray-Curtis distance = 0.23) (Fig. 4). The smallest prey (< 0.4 mm in total length) were not sampled with the 200- μ m mesh net and the comparison with sardine diet showed higher differences (Bray-Curtis distance = 0.49). The mean size of sardine prey (0.84 ± 0.43 mm) was significantly larger than the mean size of organisms sampled with the 80- μ m mesh net (0.76 ± 0.41 mm) ($H = 95.19$, $P < 0.001$). But, lower proportions of organisms > 1000 μ m were found in sardine diet and 80- μ m mesh net samples (28.6%, 25.0% of the measured organisms respectively) than in the 200- μ m mesh net samples (48.9%). Thus, both group composition and size distribution of organisms of sardine stomach content were more similar to those of the 80- μ m mesh net samples than to those of the 200- μ m mesh net. For this reason, the temporal comparison between sardine diet and plankton organism variations was focused on the 80- μ m mesh net samples.

In spite of a higher abundance of organisms sampled with the 80- μ m mesh net than with the 200- μ m mesh net, the monthly mean abundance did not significantly differ between nets ($P > 0.05$) due to the high variance of data (Fig. S4).

The monthly mean values of %IRI of sardine diet and of plankton sampled with the 80- μ m mesh net both varied between months ($\chi^2 = 1037.60$ and $\chi^2 = 4248.70$ respectively and $P < 0.001$ for both) (Fig. 5). The sardine diet appeared much more variable temporally than the zooplankton community, and the dominant groups in sardine diet were not systematically found as dominant in the water column. For example, *Microsetella/Macrosetella* spp. copepods were particularly abundant in sardine diet in March 2017 and from September 2017 to February 2018, but not in plankton tows. Similar patterns occurred with copepod nauplii, Oncaeiidae, Corycaeiidae, Centropagidae and Acartidae copepods.

The two first principal components of the PCA analysis on sardine diet explained 35.7% of the total variance of the %IRI group composition (Fig. 6a). PC1 was positively related to copepod nauplii in January 2017 and negatively to Clauso/Paracalanidae, Oncaeiidae and Oithonidae in April 2017. PC2 indicated that the sardine diet was characterized by large %IRI of bivalve larvae and Acartidae copepods in January 2018, and negatively correlated with Temoridae and Corycaeiidae in February 2017. For 80- μ m mesh net plankton samples, the PCA explained 29.5% of the total variance of the %IRI group composition (Fig. 6b). For plankton, PC1 was positively related to *Microsetella/Macrosetella* spp., Centropagidae and cypris larvae in September 2017, and negatively to Clauso/Paracalanidae in February 2017, January 2017 and March 2018. PC2 was positively related to Oncaeiidae, Calanidae, Euterpinidae, and negatively to bivalve larvae, in January 2018. The two PCAs showed a similar grouping of months with contrasting situations between a warm period (June - September) and a cold period (December - March), but

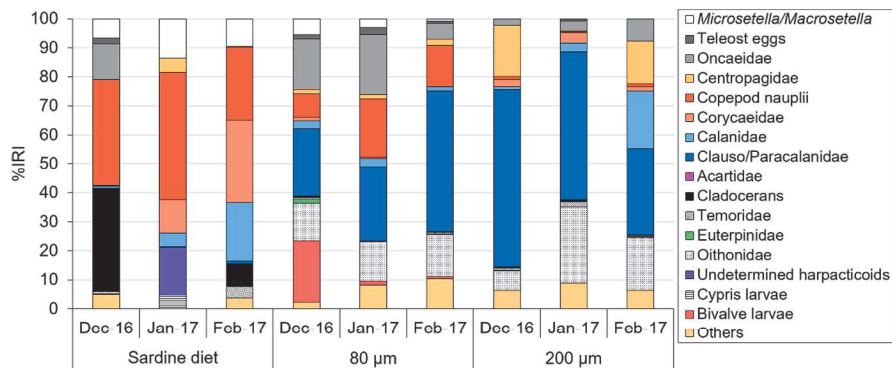


Fig. 3. Monthly averaged values of %IRI for zooplankton diet composition of *S. pilchardus* sampled in the Bay of Marseille and for community composition of zooplankton sampled with the 80- μ m and 200- μ m mesh size plankton nets at the Solemio site from December 2016 to February 2017.

Table 2

Mean (\pm SD) total length (mm) of organisms found both in *S. pilchardus* stomachs and in plankton nets (80- μ m and 200- μ m mesh size) sampled in the Bay of Marseille from December 2016 to February 2017. Superscript letters represent post-hoc groups after Kruskal-Wallis tests for the significance of differences of size of organisms found both in *S. pilchardus* stomachs and in plankton nets (80- μ m and 200- μ m mesh size). Values with similar post-hoc letters are not significantly different ($P > 0.05$).

Organisms	Group	Sardine diet	80- μ m net	200- μ m net
		mean \pm SD (mm)	mean \pm SD (mm)	mean \pm SD (mm)
Bivalve larvae	Molluscs	0.30 \pm 0.14 ^a	0.21 \pm 0.08 ^a	0.36 \pm 0.16 ^b
Copepod nauplii	Copepods	0.23 \pm 0.12 ^a	0.21 \pm 0.06 ^a	0.61
<i>Microsetella/Macrosetella</i>	Harpacticoid copepods	0.57 \pm 0.18 ^b	0.46 \pm 0.14 ^a	NA
Euterpinidae	Harpacticoid copepods	0.63 \pm 0.40 ^a	0.69 \pm 0.11 ^a	0.94
Oncaeiidae	Cyclopoid copepods	0.74 \pm 0.63 ^a	0.59 \pm 0.15 ^a	0.73 \pm 0.12 ^b
Oithonidae	Cyclopoid copepods	0.54 \pm 0.19 ^a	0.72 \pm 0.20 ^b	0.89 \pm 0.21 ^c
Cypris larvae	Cirripedes	0.88 \pm 0.22 ^a	0.82 \pm 0.11 ^b	NA
Teleost eggs	Teleosts	0.61 \pm 0.23 ^a	1.05 \pm 0.14 ^b	1.10 \pm 0.08 ^b
Temoridae	Calanoid copepods	0.77 \pm 0.42 ^a	1.09 \pm 0.23 ^a	1.00 \pm 0.33 ^a
Clauso/Paracalanidae	Calanoid copepods	0.81 \pm 0.35 ^a	1.06 \pm 0.39 ^b	1.10 \pm 0.15 ^b
Corycaeiidae	Cyclopoid copepods	1.09 \pm 0.40 ^a	0.96 \pm 0.25 ^a	1.07 \pm 0.34 ^a
Centropagidae	Calanoid copepods	1.24 \pm 0.51 ^a	1.27 \pm 0.28 ^a	1.45 \pm 0.27 ^b
Calanidae	Calanoid copepods	1.83 \pm 0.62 ^a	1.73 \pm 0.64 ^b	1.77 \pm 0.65 ^c
Mean of all measured organisms		0.69 \pm 0.44 ^a	0.84 \pm 0.43 ^b	1.11 \pm 0.46 ^c

characterized by different groups in sardine diet and plankton (Fig. 6a and 6b).

3.2. Sardine prey selectivity

The mean values of the Ivlev index of selectivity significantly differed from zero for some groups (Table 3). A positive selectivity was regularly observed for *Microsetella/Macrosetella* spp. and most of the time for Corycaeiidae copepods. In contrast, a negative selectivity occurred all the time towards Clauso/Paracalanidae and in most months for Oithonidae, copepod nauplii, Centropagidae, Calanidae, Oncaeiidae, bivalve larvae and Others (Table 3, Fig. S5). Small prey and more groups were

positively selected in April 2017 compared to August 2017, when larger groups such as Centropagidae and Acartidae were selected. Interannual differences of sardine diet were also observed, with higher proportions of *Microsetella/Macrosetella* spp. copepods ingested in winter 2017–2018 than in winter 2016–2017 (Table 3, Fig. S5). The plasticity of sardine diet was generally high ($H' = 0.61 \pm 0.45$) and varied significantly between months ($\chi^2 = 83.18$, $P < 0.001$), with low values in December 2017 and February 2018, and the highest values in March 2018 (Table 4). Strong dissimilarities (> 0.60 for Bray-Curtis distance) and thus high flexibility and differences in diet were observed between most pairs of consecutive months.

Prey size found in sardine stomach content ranged between 0.04 mm and 13.08 mm. Although the comparison of plankton size distribution in the 80- μ m mesh size net showed globally a similar unimodal distribution (Fig. 4), the monthly comparison highlighted differences with multimodal size spectra (Fig. S6). In general, a first peak emerged between 0.4 and 0.7 mm. A clear second mode of larger organisms may be identified between 1.2 and 1.6 mm in some months (May to August 2017, January 2018). At the other end of the scale, very small prey ranging from 0.1 to 0.3 mm were more frequently found in stomach content in December 2016, August 2017, January or March 2018. The mean size of prey in sardine stomach content and plankton organisms in the 80- μ m mesh size net, along with the PPSR, varied significantly between months ($H = 661.23, 304.44, 85.01$ respectively, and $P < 0.001$ for all) (Fig. 7a). Prey ranging from 0.7 to 1.1 mm were particularly frequently found in sardine diet when they were abundant in the environment such as from September to December 2017 and in February 2018. However, there was no significant correlation between the monthly mean sizes of sardine prey and plankton sampled with the 80- μ m mesh size net (Spearman's $\rho = -0.05$, $P = 0.869$).

Our results indicated that sardine was potentially able to catch prey within the whole size range of available prey in nets in any month (Fig. S7). This range appeared to be the broadest in autumn and spring, and decreased in summer, mainly related to the decrease of size and availability of large (Calanidae) and medium-sized (Centropagidae, Acartidae, Clauso/Paracalanidae and Corycaeiidae) copepods in summer. The largest and smallest organisms in 80- μ m mesh net samples were found in May 2017 (0.90 ± 0.41 mm) and in January 2017 (0.43 ± 0.24 mm) (Fig. 7a). The largest and smallest organisms in sardine diet were observed respectively in July 2017 (1.26 ± 0.18 mm) and December 2016 (0.65 ± 0.33 mm). The PPSR was also the highest in July 2017 (10.38 ± 1.65) and the lowest in December 2016 (4.75 ± 2.19) (Fig. 7b) and was positively and significantly correlated with the mean prey size ($\rho = 0.96$, $P < 0.001$).

When taxa were analyzed separately, the prey size range of a given taxon in sardine stomach content almost never corresponded to the size range of individuals of this taxon in the water column (Fig. S8). In many cases, prey individuals caught by sardine were among the largest individuals, but the opposite situation also occurred, such as for

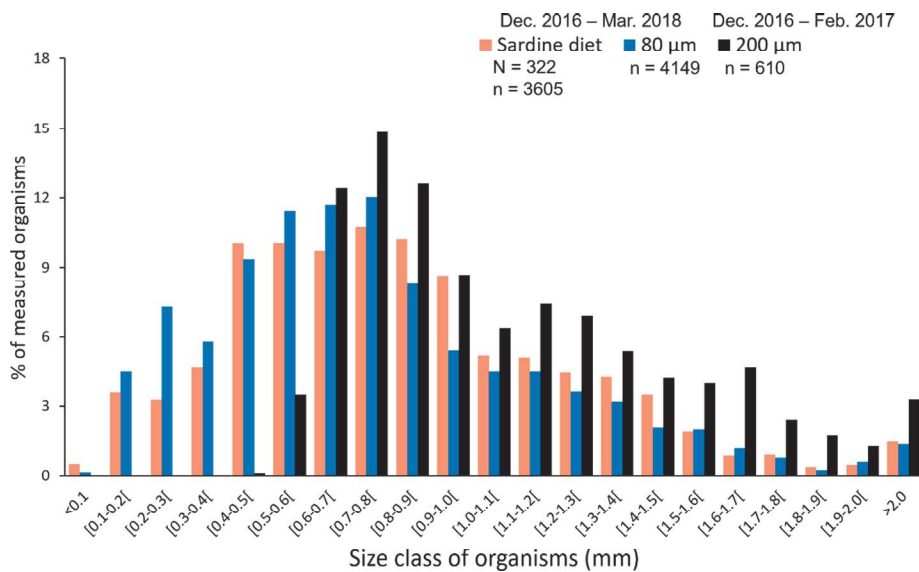


Fig. 4. Mean size distribution (0.1 mm size classes) of prey analyzed in *S. pilchardus* stomach content (orange) and of zooplankton organisms sampled with the 80- μ m mesh size plankton net over the whole study period from December 2016 to March 2018 (blue) and of zooplankton organisms sampled with the 200- μ m mesh size plankton net during the test-phase of the study, from December 2016 to February 2017 (black), in the Bay of Marseille. N = number of analyzed individuals of *S. pilchardus*, n = number of measured zooplankton organisms. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

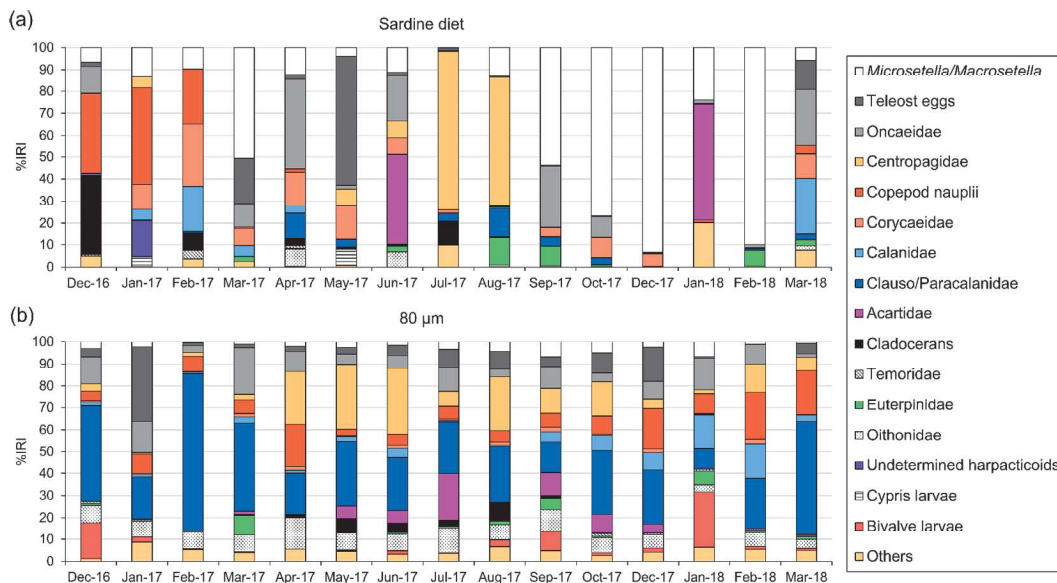


Fig. 5. Monthly mean values of %IRI for (a) diet composition of *S. pilchardus* sampled in the Bay of Marseille and (b) zooplankton community composition sampled with the 80- μ m mesh size plankton net from December 2016 to March 2018.

Oithonidae in spring (see Fig. S7 and Fig. S8).

4. Discussion

The originality of this study consists in the comparison of the temporal variation in sardine adult diet, in terms of prey group and size composition, in relation to the prey available in the environment over a 16-month period. This approach was adopted in the context of recently observed changes in the populations of *Sardina pilchardus* in the NWMS (Saraux et al. 2019) that is assumed to be related to changes in the composition of zooplankton and sardine diet.

4.1. Methodological issues and improvements

One of the main challenges of such a temporal study is related to the methodological difficulty of obtaining both plankton and sardine samples in the same place and time (see Material & Methods section). However, even if sardines and zooplankton were sampled together, it

would still be difficult to relate them because of the respective movements of water masses and sardines when feeding. In our study, sampling of these two compartments was separated by a few days in time and a few kilometers in space. The knowledge of the environmental conditions in the Bay of Marseille through the SOMLIT program and of the behavior of sardine by professional fishermen, give us confidence on the robustness of our results and cautious interpretation. The long monthly sampling period of our study (16 months), which is rare, was specifically expected to highlight the diversity of feeding conditions of sardines in this area in terms of prey species and size spectra at a seasonal scale. Most studies focusing on sardine feeding were based on oceanographic cruises in targeted areas and periods and faced similar difficulty in relating plankton to fish feeding (Costalago et al. 2012; 2014; Le Bourg et al. 2015; Bachiller et al. 2020).

Both group composition and size distribution of sardines' prey in stomach content were more similar to 80- μ m mesh net samples than those of the 200- μ m mesh net. However, by performing vertical tows with the 80- μ m mesh net, we might have underestimated some larger

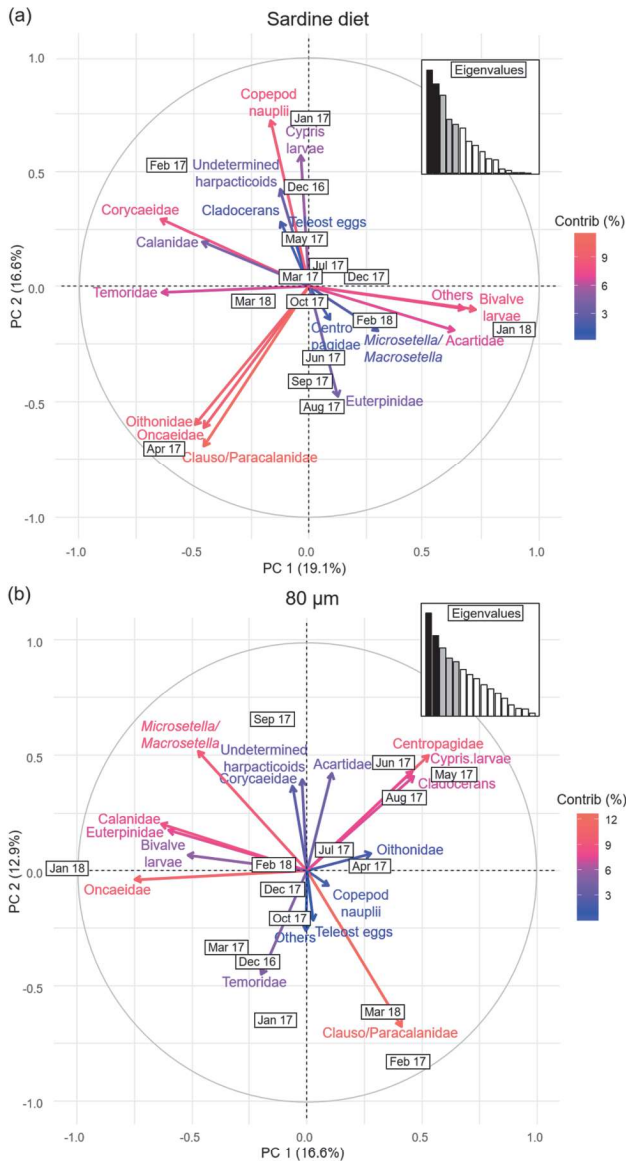


Fig. 6. Principal component analysis (PCA) of %IRI by month from December 2016 to March 2018 for (a) diet composition of *S. pilchardus* sampled in the Bay of Marseille and for (b) community composition of plankton sampled with the 80-µm mesh size plankton net at the Solemio site. Contrib (%) is the scale of the contribution values for each group.

prey such as Centropagidae copepods, which may have caused an overestimation of their electivity value. Seasonally-dependent water filtration efficiency due to possible net clogging may also have implications when extrapolating temporal patterns in community structure from abundance (Riccardi 2010; Mack et al. 2012). However, the 80-µm mesh net seemed to be the best compromise to capture the small zooplankton consumed by sardines in the Bay of Marseille and to avoid net clogging. This highlighted again the importance of the choice of mesh size of plankton nets to accurately compare the diet of planktivorous fish species with community composition of zooplankton available in the environment. In areas with a wide size spectrum of prey size and for a wide size range of predators, it may be useful to combine multiple nets (Costalago and Palomera 2014; Bachiller et al. 2020). A possible method to better relate sardine and zooplankton sampling is to simultaneously use acoustic systems to detect fish feeding depth (before catching them) and multiple nets stratified-horizontal tows (such as the multiple plankton sampler Hydro-Bios) to target zooplankton at the

Table 3 Monthly mean Ivlev's electivity index value of *S. pilchardus* prey groups in relation to zooplankton organisms sampled with 80-µm mesh size plankton net in the Bay of Marseille from December 2016 to March 2018. Capital letters are initials of months. Values are presented by decreasing order of mean %IRI of groups (***P < 0.0001, **P < 0.001, *P < 0.01, *P < 0.05).

Year	Month	2018												Mean ± SD		
		D	D	M	A	M	J	J	A	S	O	D	J			
Microsetella/Macrosetella	Teleost eggs	0.08	0.18	-1.00	0.63	0.71	-0.94	0.23	0.61	0.71	0.91	0.36	0.96	0.66	0.49 ± 0.49*	
	Oncaeidae	-0.17	-1.00	-0.96	-1.00	0.68	-0.17	-0.27	-0.05	-0.71	-0.86	-1.00	-1.00	1.00	0.83	0.02 ± 0.81
Centropagidae	Copepod nauplii	-1.00	0.53	-0.96	0.53	-0.52	0.86	0.84	-1.00	0.36	-0.96	-1.00	-1.00	-0.77	0.84	-0.35 ± 0.64*
	Corycaeidae	0.63	0.37	0.28	0.37	-0.40	0.86	0.84	-1.00	0.21	-1.00	-1.00	-1.00	-1.00	-0.82	-0.57 ± 0.71*
Calanidae	Copepod nauplii	-0.58	0.91	0.99	0.24	-0.82	-0.79	-0.82	-1.00	0.00	-1.00	-1.00	-1.00	-1.00	0.97	-0.61 ± 0.60*
	Corycaeidae	-0.59	0.91	0.99	0.24	-0.82	-0.79	-0.82	-1.00	0.00	-1.00	-1.00	-1.00	-1.00	0.80	0.46 ± 0.67*
Acartidae	Cladocerans	-0.99	-1.00	-0.97	0.00	-0.77	-0.94	-0.02	-0.77	0.91	-0.94	-1.00	-1.00	-1.00	-0.83	-0.48 ± 0.66'
	Teleost eggs	-1.00	0.00	-1.00	0.00	-0.94	-0.07	-0.77	-0.94	-1.00	-1.00	-1.00	-1.00	-1.00	-0.60	-0.73 ± 0.42**
Temoridae	Cladocerans	0.98	-0.97	0.94	0.05	0.76	0.83	1.00	1.00	0.44	-1.00	-1.00	-1.00	0.00	0.60	-0.40 ± 0.85
	Euterpinidae	-0.97	-0.62	0.79	0.00	0.06	0.84	1.00	1.00	-0.38	-1.00	-1.00	-1.00	0.00	0.00	-0.25 ± 0.81
Oithonidae	Undetermined harpacticoids	-1.00	-0.90	-1.00	0.00	0.45	-0.82	-0.88	-0.77	0.00	-1.00	-1.00	-1.00	0.58	0.00	-0.27 ± 0.75
	Cypris larvae	0.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	-0.07	-0.08	-0.91	-1.00	0.00	0.00	-0.35 ± 0.63
Bivalvia larvae	Undetermined harpacticoids	1.00	1.00	1.00	1.00	0.96	1.00	1.00	1.00	-0.93	-0.99	-1.00	-1.00	0.00	0.00	-0.84 ± 0.31**
	Cypris larvae	-1.00	-1.00	-1.00	-1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13 ± 0.35
Others	Bivalvia larvae	0.35	0.35	-1.00	-0.97	-1.00	-1.00	-1.00	-1.00	0.00	0.00	-0.89	-1.00	0.00	0.00	0.20 ± 0.67
	Others	0.35	0.35	-1.00	-0.97	-1.00	-1.00	-1.00	-1.00	0.64	-0.97	-0.92	-0.93	-1.00	-0.10	-0.66 ± 0.61*

Table 4

Monthly mean Shannon-Wiener diversity index (H') value of *S. pilchardus* diet and Bray-Curtis distance of pair-wise comparison of diet composition between months for samples collected in the Bay of Marseille from December 2016 to March 2018. Superscript letters represent post-hoc groups after Kruskal-Wallis test. Values with similar letters are not significantly different ($P > 0.05$). Bray-Curtis distance between consecutive months > 0.60 are marked in bold.

Month	H' index	Dec-16	Jan-17	Feb-17	Mar-17	Apr-17	May-17	Jun-17	Jul-17	Aug-17	Sep-17	Oct-17	Dec-17	Jan-18	Feb-18
Dec-16	0.58 ± 0.46^{ac}														
Jan-17	0.60 ± 0.40^{ac}	0.55													
Feb-17	0.78 ± 0.39^a	0.55	0.49												
Mar-17	0.58 ± 0.41^{ac}	0.77	0.73	0.74											
Apr-17	0.71 ± 0.39^a	0.72	0.71	0.65	0.62										
May-17	0.43 ± 0.41^{cd}	0.90	0.74	0.78	0.65	0.72									
Jun-17	0.77 ± 0.54^a	0.77	0.76	0.81	0.66	0.50	0.76								
Jul-17	0.62 ± 0.36^{ac}	0.82	0.93	0.86	0.94	0.89	0.84	0.88							
Aug-17	0.85 ± 0.47^{ab}	0.90	0.81	0.88	0.83	0.72	0.83	0.76	0.36						
Sep-17	0.74 ± 0.34^a	0.79	0.82	0.85	0.32	0.50	0.86	0.61	0.94	0.72					
Oct-17	0.50 ± 0.37^{acd}	0.83	0.77	0.80	0.31	0.65	0.81	0.70	0.94	0.82	0.29				
Dec-17	0.14 ± 0.29^d	0.92	0.81	0.84	0.43	0.81	0.90	0.83	0.97	0.86	0.42	0.17			
Jan-18	0.58 ± 0.35^{ac}	0.87	0.86	0.86	0.72	0.85	0.93	0.46	0.89	0.87	0.74	0.74	0.75		
Feb-18	0.14 ± 0.21^d	0.91	0.86	0.89	0.45	0.85	0.93	0.85	0.98	0.79	0.37	0.20	0.09	0.74	
Mar-18	1.21 ± 0.30^b	0.69	0.74	0.54	0.52	0.45	0.65	0.59	0.86	0.85	0.58	0.71	0.88	0.84	0.89

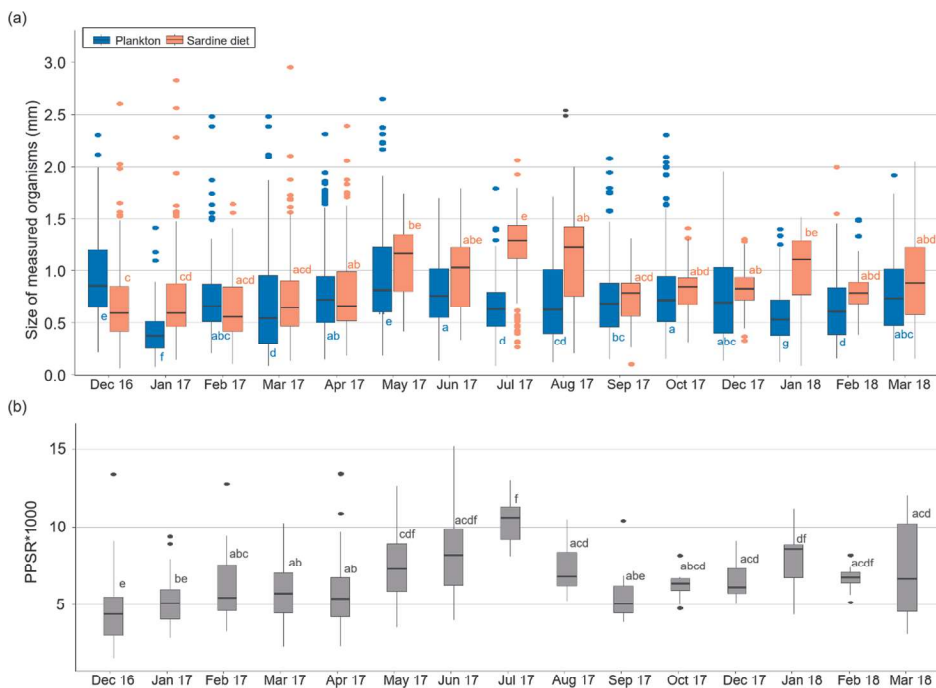


Fig. 7. (a) Comparative boxplots of total length (median \pm interquartile range) of all zooplankton organisms sampled with the 80- μ m mesh-size plankton net (blue) and of all zooplankton prey found in *S. pilchardus* stomachs (orange), and (b) Boxplots of PPSR (values multiplied by 1000) for every month from December 2016 to March 2018. Superscript letters represent groups of post-hoc Dunn's tests after analyses of variance. Values with similar letters are not significantly different ($P > 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

same depth.

If the organisms can be well-preserved in stomach content, for example by injection of formaldehyde into the abdominal cavity immediately after capture to stop the digestion, it is suggested that future studies use the automatic imagery systems FlowCAM and ZOO-SCAN to obtain other biometric measurements in addition to maximum length of each prey, enabling more precise comparison with zooplankton organisms sampled with nets. Large or soft prey are often broken or in advanced digestive stage such as chaetognaths, siphonophores, salps, fish eggs, cladocerans and even certain copepod species (e. g. Oithonidae) and their identification can be harder than that of the small hard copepods (Sutela and Huusko 2000; Bachiller et al. 2020). This could have an effect on interpretation of selective feeding data using the Ivlev electivity index. Future studies may use other methods, such as DNA barcoding, which may complete the prey identification (Bachiller et al. 2020).

4.2. Sardine diet characterization

Among the 29 prey groups identified in sardine diet in the Bay of Marseille over a 16-month period, the most consumed were *Microsetella/Macrosetella* spp., Oncaeidae, Centropagidae, copepod nauplii and Corycaeidae copepods. A high similarity was observed with the diet of sardine of the Gulf of Lion (Costalago and Palomera 2014; Le Bourg et al. 2015; Bachiller et al. 2020). The diversity of available prey in the Bay of Marseille appeared to be as high as in the diverse planktonic habitats of the Gulf of Lion (Espinasse et al. 2014). This may be linked to the complex hydrology of this area and the various sources of particulate organic matter (Cresson et al. 2012; Bănarau et al. 2014; Frayssse et al. 2014; Millet et al. 2018; Chen et al. 2019). However, some differences were evidenced, in particular, the low importance of cladocerans as prey in the Bay of Marseille, in agreement with the results of Le Bourg et al. (2015) and contrary to those of Costalago and Palomera (2014) in the Gulf of Lion. However, using DNA metabarcoding analyses, Bachiller et al. (2020) found a frequency of occurrence of 12% for cladocerans in the diet of sardine from the western Gulf of Lion, while they were not

detected visually in stomach contents. In the Bay of Marseille, the consumption of cladocerans may be related to their sporadic appearance linked to Rhone River plume intrusions, as shown by Villate et al. (2014) for the Ebro river plume in the Catalan Sea and also observed in the Bay of Marseille by M. Pagano (pers. comm.), but not optimally recorded by our plankton sampling schedule.

The size range of sardine caught in the Bay of Marseille (12.80 ± 1.40 cm) is similar to that observed in the Gulf of Lion, but smaller than in the Atlantic and the southern Mediterranean Sea (Table S2 and references therein). As prey size may differ according to predator size, diet differences among sardine size classes may be observed depending on prey patchiness, and the swimming capacity and morphological particularities of fish size classes. Previous studies in the Atlantic showed that adult sardine may consume more phytoplankton and smaller prey, related to an increasing number of gill rakers and denticles of their filtering apparatus with growth, than Mediterranean sardine, which have fewer, more widely separated gill rakers, potentially inducing a more pronounced zooplanktivorous diet (Andreu 1969; Bode et al. 2004; van der Lingen et al. 2009; Costalago et al. 2015). However, in the Bay of Marseille, prey size distribution and composition of sardine diet did not differ among size classes during the whole sampling period. This may reflect the slower growth and probably the relatively poorer feeding conditions of sardine populations in this region compared to those of southern parts of the NWMS (Bachiller et al. 2020). In the Bay of Marseille, sardine ingested high proportions ($\geq 60\%$) of prey from 0.4 to 1.0 mm in size and only small proportions of prey > 1 mm, and PPSR were in the same range as those estimated by Le Bourg et al. (2015) in the Gulf of Lion. The selection of *Microsetella/Macrosetella* spp. and Corycaeidae copepod groups by sardine observed in this study was also documented in other studies (Costalago and Palomera 2014; Le Bourg et al. 2015), as well as teleost eggs selection (Garrido et al. 2007; 2008; Costalago et al. 2015). Harpacticoid and cyclopoid copepods are known to be often associated with marine snow or phytoplankton filaments (Carloti et al., 2018; Kiørboe, 2000; Koski et al., 2020; Uye et al., 2002 and references therein). The high contribution of these small copepods to sardine diet found here may be linked to the high load of aggregates in the Bay of Marseille due to frequent resuspension of supra-benthic detrital matter and occasional Rhone river input (Bănanu et al. 2014; Espinasse et al. 2014; Millet et al. 2018; Chen et al. 2019), particularly in period of filter-feeding mode for sardine (Nikolioudakis et al. 2012 and references therein).

The high diversity of sardine diet may be also related to their large prey size range as highlighted in several studies comparing sardine diet to those of other small pelagic fish species (van der Lingen et al. 2009; Nikolioudakis et al. 2012; Canales et al. 2016). Larger individuals may ingest greater numbers of larger prey as they grow, due to increasing energy requirements (Costalago et al. 2012). Different studies on biochemical components in zooplankton (Hagen and Auel 2001; Lee et al. 2006) indicated an increase in protein and lipid concentrations, and thus of energy, in larger organisms (Harmelin-Vivien et al. 2019; Chen et al., 2019). Larger calanoid copepods such as Calanidae have higher energy content than smaller ones such as Centropagidae, Temoridae and Acartidae (Ventura 2006; Dessier et al. 2018). Thus, the abundant large Clauso/Paracalanidae copepods were expected to occur in high numbers in sardine stomachs, which was not the case. The negative values of Ivlev selectivity index for these groups could suggest that their capture was too costly energetically, in accordance with the observations of Nikolioudakis et al. (2012, and references therein) in the Northern Aegean Sea. Teleost egg consumption by sardine has been often observed and is considered as a self-regulatory mechanism when it comes to the eggs of sardine itself and/or to compensate their nutritional requirements during food shortage (Garrido et al. 2008; Le Bourg et al. 2015). The consumption of eggs of other species sharing their niche may also be the result of intraguild predation and a mechanism in species alternation (Irigoiien and de Roos 2011; Bachiller et al. 2015; Peck et al. 2020). The seasonality of teleost egg consumption by sardine, positively

selected in spring (from March to June), may help to better understand the dynamics between fish species in the Bay of Marseille.

4.3. Temporal variability and plasticity of sardine diet

A high diet plasticity and flexibility was observed for sardine in the Bay of Marseille highlighted by the wide diversity of consumed prey and the high temporal variability, both between months and between years. In addition, prey selectivity was suggested by the comparison of stomach contents and zooplankton in nets. PCAs showed that month grouping was similar in sardine diet and zooplankton, with contrasting warm (June to September) and cold (December to March) periods, but characterized by different plankton groups. This means that seasonality influenced similarly plankton communities and sardine diet, but that sardine did not select systematically the most abundant groups observed in the environment. Winter and spring appeared as key periods of higher feeding intensity for sardine, which could be related to peaks of the energy content of zooplankton size classes (high abundance of small size groups in spring, and high abundance of large size groups in winter) (Chen et al. 2019). These peaks were probably related to rapid reproduction processes of copepods linked to more productive conditions (Table S3), due to the environmental and hydrological conditions affecting zooplankton composition in the bay at these periods (Chen et al. 2019). Previous results indicated that sardine diet in the Mediterranean Sea was based on larger prey such as large calanoid copepods and other crustaceans (mainly decapod larvae) (Nikolioudakis et al. 2012; Costalago and Palomera 2014), while our results indicated that it was now mainly based on small organisms such as *Microsetella/Macrosetella* spp. A decrease in the abundance of large energy-rich organisms in plankton community might have occurred in recent years, with a potential negative impact on the body condition of sardine, leading to their smaller body size and hence the lower biomass observed recently in the Gulf of Lion (Le Bourg et al., 2015; Saraux et al. 2019).

Our temporal approach suggests the existence of two feeding modes in sardine with a shift from filtering to selective particulate-feeding depending on prey concentration and size spectrum (van der Lingen 1994). When the prey concentration declines, sardine switch from passive filtration to active selective predation of prey larger than a given size threshold, which can vary with species and season, or at any time depending on the density and aggregation of prey (van der Lingen 1994; Garrido et al. 2007). However, particulate-feeding is more energy-demanding than filter-feeding due to the frequent changes in swimming direction and velocity involved in hunting (van der Lingen 1994). In the Bay of Marseille, the zooplankton size spectrum was wide but dominated by small forms during productive periods (spring and autumn/winter) (Fig. 8C), and small organisms dominated then also in stomach contents (Fig. 8A). This could be related to the dominance of filter-feeding mode, more profitable in these circumstances (van der Lingen 1994). Filter-feeding mode was also favored in winter by water mixing, which limited stratification and prey aggregation (Donoso et al. 2017). Inversely, in low production periods (summer in our study) a narrower zooplankton size range was observed in the Bay of Marseille (Fig. 8D), as observed elsewhere (Halsband-Lenk 2001; Wesche et al. 2007; Arima et al. 2014), and a more balanced distribution between small, medium and large zooplankton prey was recorded in sardine stomach contents (Fig. 8B). The aggregation of zooplanktonic prey in the stratified water column made particulate-feeding more profitable (van der Lingen 1994). Consequently, it is not only size that matters for sardine feeding, but the combination of taxonomic identity, escape behavior (Pasternak et al. 2006), energy value (Lee et al. 2006; Nikolioudakis et al. 2012), vulnerability of prey (Strickler et al. 2005), and the effect of predator schooling behavior on prey (Eggers 2011). To sum up, filter-feeding may be the dominant feeding mode in sardine but the use of particulate-feeding may be seasonally dependent according to prey density and size (Garrido et al. 2007).

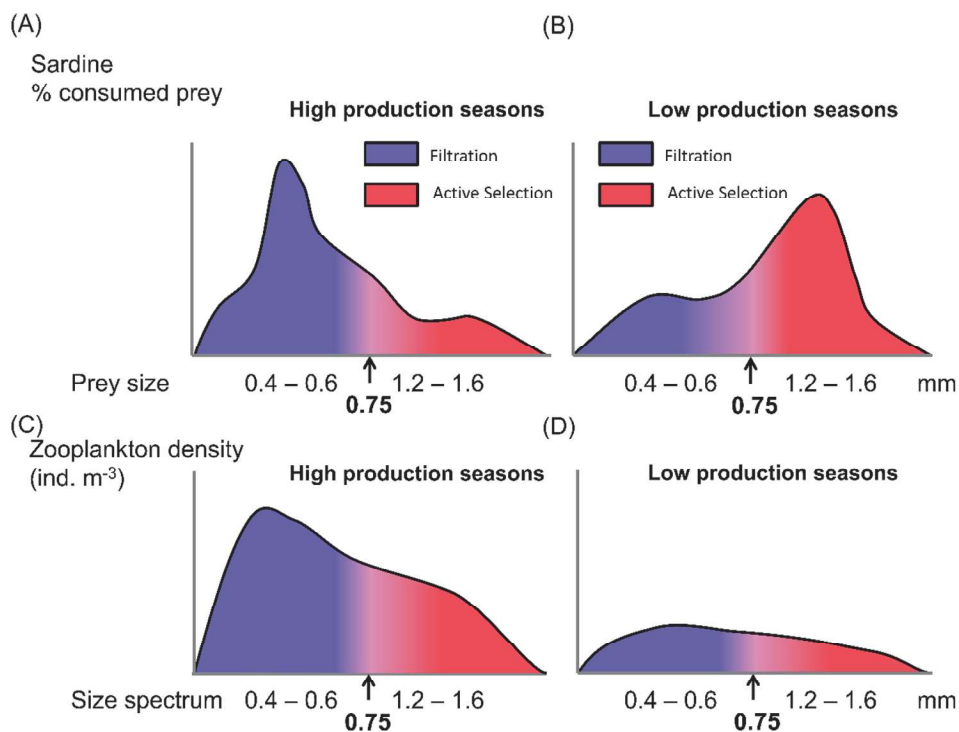


Fig. 8. Conceptualization of sardine dominant feeding modes related to zooplankton abundance size spectra for different productivity state of the pelagic ecosystem. A and C: high production seasons, B and D: low production seasons. A and B: prey abundance size spectra in sardine stomachs, C and D: zooplankton abundance size spectra in field-caught plankton. 0.75 mm plankton size threshold: filtration mode dominates when smaller plankton size classes are abundant, active selection mode dominates for larger plankton size classes during low production seasons.

5. Conclusion

The taxonomic and size composition of sardine stomach content was analyzed monthly during a 16-month period and compared to those of zooplankton sampled with plankton nets at the Solemio site in the Bay of Marseille, NW Mediterranean Sea. We observed that (1) sardine diet differed between months in terms of prey groups and size; (2) sardine may have selected certain prey groups such as *Microsetella/Macrosetella* spp. and Corycaeidae; (3) rather small prey ranging from 400 to 1000 μm in size were consumed; (4) sardine consumed the largest prey in summer; (5) the taxonomic and size composition of sardine stomach content were more similar to those of plankton sampled with the 80- μm mesh net than with the 200- μm mesh net. This underlines the importance of an appropriate methodological choice.

The results of our study indicate that sardine has high diet plasticity and flexibility, being able to adapt its feeding mode (filter-feeding vs selective particulate-feeding) in order to consume a broad diversity of prey of a wide size spectrum and that it generally chooses the most interesting sizes in terms of energy balance, depending on the available prey size spectrum (Chen et al. 2019).

The seasonal and inter-annual variability shown in this study may justify the interest of a longer monitoring period to provide a basis for linking the variability of the zooplankton community in terms of taxonomic and size composition to sardine diet and to better understand their trophic dynamics. Such results may serve as a database for modelling studies and contribute to a better understanding of pelagic food web functioning in the North-Western Mediterranean Sea.

References in tables and supplementary material

(Hopkins 1966; Dumont et al. 1975; Fonda Umani et al. 1979; Mauchline 1998; Lindley et al. 1999; Satapoomin 1999; Ara 2001; van der Lingen 2002; Pitois and Fox 2006; La Mesa et al. 2008; Garrido et al. 2008; Borme et al. 2009; Delpy 2013; Costalago and Palomera 2014; Dessier 2015; Le Bourg et al. 2015; Bachiller et al. 2015; 2020; Albo-Puigserver et al. 2020).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pocan.2021.102617>.

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