Feeding habits and helminth parasites of sardine (S. pilchardus) and anchovy (E. encrasicolus) in the Adriatic Sea

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Research Article

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Abstract

We examined the stomach contents of two of the most economically and ecologically important small pelagic fish species, the sardine, *Sardina pilchardus* and the anchovy, *Engraulis encrasicolus*, obtained monthly from commercial purse-seine catches operating on Croatian fishing grounds during a one-year period (January–December, 2011). Both species generally showed a similar diet, with copepod and decapod larvae as dominant prey groups. The composition of anchovy and sardine stomach contents was not size- or sex-related, but throughout the year, a significant difference in diet composition was observed for each species as well as between species. Two gastrointerstinal helminths; the digenean *Parahemiurus merus* and nematode *Hysterothylacium aduncum*, were recorded during the stomach content analysis. Differences in population dynamics between two parasites are congruent with differences in the prey composition of sardine and anchovy, reflecting fine-tuned interactions in the trophic web between parasites and intermediate or paratenic hosts included in the sardine and anchovy diet.

Keywords: Diet composition, stomach contents, gastrointerstinal helminths, small pelagic fish, Mediterranean.

Introduction

Small pelagic fish species are economically important components of many marine ecosystems, which are also crucial from an ecological point of view, as they enable the transfer of energy from lower to higher trophic levels (Cury *et al*., 2000). The sardine *Sardina pilchardus* and the anchovy *Engraulis encrasicolus*, two of the most abundant small pelagic fish species, are widely distributed throughout the Mediterranean basin and its adjacent seas, as well as in the north-eastern Atlantic (Whitehead *et al*., 1988). Both of them are short-lived and fast-growing fish that migrate towards coastal waters for spawning, where they release their spawning products multiple times. The spawning season of both species is protracted, thus in the Adriatic Sea, sardine and anchovy tend to spawn from October to April (Sinovčić *et al*., 2008) and from April to September (Sinovčić & Zorica, 2006), respectively. With time, significant alternations in biomass of sardine and anchovy catches have been noted, not only in the Adriatic Sea, but also worldwide. Recently, these stock-size fluctuations have attracted scientific attention and many studies that have taken an ecosystem-based approach have been performed, which suggest that food limitation is one of the processes that might cause these alternations in abundance (Cury *et al*., 2000; Agostini & Bakun, 2002; Lloret *et al*., 2004; Santojanni *et al*., 2006; McLeod *et al*., 2012; Zorica *et al*., 2013).

Fish ecological traits, such as size, age, habitat, diet, trophic level, population size, depth distribution and geo-graphical range, strongly affect parasite assemblages that aggregate over time in fish hosts, producing a highly complex and dynamic ecological interaction (Marcogliese & Cone, 1997; Poulin & Morand, 2000; Timi *et al*. 2010). Therefore, insight into the composition of the parasite, communities in fish that act as intermediate, paratenic or final hosts, can serve to elucidate intricate patterns in the host ecology. Moreover, gastrointestinal helminth parasites have been recognised as an excellent tool to elucidate long-term trends in trophic interactions and ontogenetic or seasonal shifts in host diet (Marcogliese & Cone, 1997; Marcogliese, 2003, 2004; Petrić *et al*., 2011). In the Adriatic Sea, few studies have assessed the occurrence of helminths in sardine and anchovy (Paradižnik & Radujković, 2007; Mladineo *et al*., 2012), and the composition of these small pelagic fish parasite communities is practically unknown.

Sardine and anchovy are highly important renewable resources in the Adriatic Sea, which play a significant trophic role in the marine system, and consequently will always represent an important focus of ecological studies. Morello Betula & Arneri (2009) published an extensive review on sardine and anchovy populations in the Adriatic Sea and emphasised the future research needs that are important to elucidate the remaining questions in species ecology. One of the crucial gaps in the ecological knowledge of both species is the qualitative and quantitative characteristics of fish feeding habits. Therefore, the objectives of this study were (1) to investigate and compare the diet composition of two commercially very important small pelagic fish species on

the basis of stomach content examination, (2) to analyse potential size- and sex-related changes in sardine and anchovy diet over time and (3) to reveal gastrointerstinal parasite composition.

Material and Methods

Study area and environmental conditions

The Adriatic Sea is the northernmost semi-enclosed basin in the Mediterranean Sea with a cyclonic circulation. The cyclonic circulation pathway is caused by estuarine conditions, which are regulated by the northern Adriatic rivers (Cushman-Roisin *et al*., 2001) and local winds (Orlić *et al.,* 1992). Along the western Adriatic coast, the outflowing West Adriatic Current is present, whereas on its eastern side, the East Adriatic Current (Orlić *et al*., 2007) brings Levantine Intermediate water from the Ionian and Levantine Seas (Robinson *et al*., 1991; Malanotte-Rizzoli *et al*., 1997). Levantine Intermediate water can flood the Adriatic periodically in so-called ingression years (Buljan, 1953), causing increases in salinity, temperature and nutrients in the intermediate layer (Buljan & Zore-Armanda, 1979; Tziperman & Malanotte-Rizzoli, 1991; Vilibić & Orlić, 2001, 2002). During the winter, the water column is homogenised due to substantial wind-mixing and winter-surface cooling, whereas in summer, when wind mixing is very mild, Adriatic waters become highly stratified (Buljan & Zore-Armanda, 1976).

As a whole, the Adriatic is a temperate sea, with temperatures even in the deepest layers generally above 10°C. Salinity is relatively high, ranging between 38.4 and 38.9 in the open southern part and the lowest values are found in the north, close to the Po river delta. In the South Adriatic Pit, the highest salinity is recorded in the intermediate layer. (Zore-Armanda *et al*., 1999; Cushman-Roisin *et al.,* 2001).

The Adriatic Sea, especially its northern part, is one of the richest fishing grounds in the Mediterranean (Cingolani *et al*., 1996). The river inflows over the shallow northern Adriatic shelf, coupled with the mixing of bottom sediments, enable a high productivity of this area. The central and southern Adriatic are less productive (Fonda Umani, 1998), but their exposure to the influence from the north Adriatic via the outflowing West Adriatic Current, as well as periodically strong influences of Mediterranean water also enhance productivity in these areas (Zore-Armanda, 1984; Hure & Kršinić, 1998).

Considering that small pelagic fish forage on plankton during their whole life cycle, and that mesozooplankton is often cited as the significant prey (van der Lingen, 2002; Espinoza & Bertrand, 2008; Garrido *et al.,* 2008), this size fraction of the zooplankton community is especially targeted in fisheries-related research. In the Adriatic Sea, the mesozooplankton is generally dominated by copepod crustaceans, especially in the open sea. Other zooplankton groups, such as cladocerans and miscellaneous meroplankton larvae (e.g. bivalves, gastropods, polychaets, decapods), can be highly important in neritic environments, whereas gelatinous plankton shows the highest variability in occurrence and abundance (Batistić *et al*., 2004; Vidjak *et al*., 2006, 2012). Within the copepod community, the population structure and the relative importance of individual species point to a characteristic high ranking of small- and medium-sized taxa, in particular from the genera *Acartia*, *Temora, Centropages, Clausocalanus, Oithona* and *Oncaea* (Kršinić & Grbec, 2012; Vidjak *et al*., 2012).

Generally, zooplankton abundance and biomass decrease from the northern to southern part, and from coastal to open waters (Fonda Umani, 1996). The zones of high plankton productivity are linked to wide areas under pronounced river influences (northern Adriatic and SW coast) or eutrophication effects (coastal bays in the vicinity of urban settlements). The open central part of the Adriatic Sea is the least homogeneous in terms of zooplankton productivity potential, where the influences of a multitude of environmental parameters (topography and currents) result in productive micro-locations such as Palagruža and Blitvenica regions, which are connected to an abundant fisheries potential (Hure & Kršinić, 1998).

Sampling methods

Sardine and anchovy specimens were collected along the eastern Adriatic Sea, on the Croatian fishing ground in 2011, within the framework of the National Monitoring Project ("Biodiversity and Management of Pelagic and Demersal Resources of the Adriatic Sea"). Fish were caught during the night with commercial purse seines (mesh size of 10 mm), operating with artificial light.

Fish samples were collected randomly each month during the studied period (January 2011–December 2011) on Croatian fishing ground. Immediately after landing, the total length (TL, cm) of one box of sardine and anchovy specimens (approximately 7–8 kg) was recorded onboard. In addition, a subsample (at least 30 specimens of each species per season; Table 1) of sardine and anchovy specimens was used for stomach content analysis. The subsample comprised individuals whose visceral cavity was carefully opened prior to preservation in 10% buffered formalin. In the laboratory, each individual from a subsample was measured (TL, cm) to the nearest 1 mm and weighed (total body weight W, g) to a precision of 0.01 g. The sex of the specimens was determined macroscopically. Stomachs were removed and measured to an accuracy of 0.01 g before and after (total weight of full W_{sf}, g; and empty stomach W_{se}, g) the prey items were carefully extracted. Prey items were identified under a stereomicroscope (magnification 40-80x) to the taxonomic level of class or family and were counted.

Gastrointestinal helminths were isolated from the stomach contents, carefully washed with filtered seawater, fixed in 70% ethanol and counted. Using appropriate identification keys, helminthes were identified as *Parahemiu-*

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rus merus (Hemiuridae, Digenea) and the third larval stage of *Hysterothylacium aduncum* (Nematoda, Anisakidae) (Gibson *et al.*, 2001; Grabda, 1976). After morphological identification, *H. aduncum* larvae were confirmed using molecular tools; genomic DNA was isolated and a ~600 bp-long fragment of the mitochondrial cytochrome oxidase 2 (cox2) locus was amplified as previously described (Nadler & Hudspeth, 2000) from five larvae from each host. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and sequenced in both directions using an ABI 3100 automatic DNA sequencer (Applied Biosystems) and the ABI PRISM BigDye Terminator Cycle Sequencing Kit. Sequences were aligned with other anisakid sequences stored in GenBank (http://www.ncbi.nlm.nih.gov/Genbank/GenbankSearch.html: *Anisakis simplex* s. s. (DQ116426), *A. pegreffii* (DQ116428), *A. simplex* C (DQ116429), *A. typica* (DQ116427), *A. ziphidarum* (DQ116430), *A. physeteris* (DQ116432), *A. brevispiculata* (DQ116433), *A. paggiae* (DQ116434), *A. nascettii* (DQ116431) (as reported in Mattiucci *et al.,* 2009) and *H. aduncum* (JQ934891, JQ934892)) using Clustal X implemented in MEGA 5.05 software (Tamura *et al.*, 2011) using default parameters and were further verified by GBlocks (http://molevol.cmima.csic.es/castresana/Gblocks.html). The cytochrome oxidase 2 sequence was added to GenBank and was assigned the accession number KM065512.

Phylogenetic reconstruction was performed using the Neighbour-Joining (NJ) method implemented in Mega 5.05 and testing the bootstrap value at 2,000 replications, using the TrN+G (Tamura & Nei, 1993) evolution model of nucleotide substitution with the gamma parameter selected in jModelTest 0.1.1 (Posada, 2008). An unrooted consensus tree was constructed from the tree output file produced in MEGA and was visualised using TreeExplorer (MEGA 5.05).

Fish were divided into size classes: small (TL < 12.9 cm) and medium anchovies (TL > 13.0 cm); small (12.0 < TL < 14.9 cm), medium (15.0 < TL < 16.9 cm) and large sardines (17.0 < TL < 19.9 cm). To assess the diet composition of each species by sex, size classes and season, the following indices were calculated:

The fullness index (%Jr):

%Jr = (Wp/W) × 100, where Wp is the weight of prey items calculated as a discrepancy between the weights of full and empty stomachs (W_{sf}-W_{se}); W refers to a total body weight.

The vacuity index (%V) was calculated as:

%V = E/N × 100, where E is the number of empty stomachs and N, the total number of stomachs analysed.

The frequency of occurrence (%F):

%F = n/N × 100, where n is the number of stomachs containing a certain prey and N is the total number of analysed stomachs containing any kind of prey.

The abundance (%N):

%N = np/Np × 100, where np is the number of prey specimens in a specific group and Np is the number of all determined prey groups.

Statistical analyses

Data on the stomach contents for the investigated small pelagic fish species were log (x+1)-transformed, and a Bray–Curtis similarity matrix was constructed using the PRIMER software package (Plymouth Marine Laboratories, UK; Clarke, 1993; Clarke & Warwick, 1994). A one-way ANOSIM test was applied to sex- and length-class differences between each species (Clarke & Warwick, 1994).

To estimate and compare the diet characteristics between anchovy and sardine, some descriptive indices were calculated. The biodiversity of prey was calculated using the Shannon–Wiener index (H′), which considers both the specific richness and the number of specimens for each species.

$$H' = -\sum p_i \log 2 p_i$$

where p_i is the percentage of the number of the prey i found in the diet.

The equitability (E) provides the comparison between the percentage by number of each prey and varied from 0 to 1; a value of 1 indicates that each prey had the same percentage by number.

$$E = H'/\log 2R$$

The food overlap of sardine and anchovy specimens was calculated using Schoener's index (Schoener, 1968) (D).

$D = 1 - 0.5\sum|P_{sardine,i} - P_{anchovy,i}|$, where $P_{sardine,i}$ and $P_{anchovy,i}$ are the respective proportions for sardine and anchovy for the i-th prey item. A range between 0.25 and 0.74 was considered moderate, whereas values >0.74 were defined as indicating substantial overlap (Pedersen, 1999).

A two-way crossed ANOSIM test was applied to test for differences in stomach contents between different species and seasons, whereas SIMPER analysis was performed to highlight prey items that contributed to seasonal differences in the diet of anchovy and sardine.

Parasite prevalence and abundance were calculated according to Bush *et al.* (1997). To assess the population dynamic of two isolated parasitic species over one year, Quantitative Parasitology 3.0 software (QP3.0) (Reiczigel & Rozsa, 2005) was used. Sterne's exact 95% confidence limits (CL) were calculated for prevalence, bootstrap 95% confidence limits (number of bootstrap replications = 2,000) for mean abundance and mean intensity, and the exponent of the negative binomial (k) (Bliss and Fisher, 1953) for parasite skewness. To test whether the prevalence, mean abundance and intensity of two parasite species differed between anchovy and sardine, the bootstrap *t*-test was used. Differences among prevalences were tested by unconditional tests, both incorporated within the QP3.0. Brunner–Munzel test of stochastic equality of intensity distributions (number of bootstrap replications = 2,000) and a two-sample comparison of mean crowding (97.5% confidence intervals used to observe potential overlap) was also used in QP3.0.

Results

Anchovy

Length frequency distribution

The total length of anchovy specimens collected for stomach content analysis (N = 295) ranged from 9.9 to 17.1 cm, with a mean of 13.58 ± 1.27 cm, and did not differ significantly (Wald–Wolfowitz Runs Test; Z = -1.165, p = 0.243) from that obtained in catches (from 9.0 to 18.0 cm; mean of 13.83 ± 0.54 cm) over the investigated year. The weight of anchovy sampled for diet composition ranged from 4.91 to 34.29 g with a mean of 15.34 ± 4.93 g. The overall sex ratio of the investigated subsamples for stomach content was 0.86; the proportion of females was predominant over that of males but was not statistically significantly different (χ^2 = 1.704, df = 1, p = 0.191). Nevertheless, similarity was observed between sexes regarding length (♂: N = 131; 9.9 < TL < 16.8 cm; mean ± SD: 13.62 ± 1.28 cm; ♀: N = 153; 9.9 < TL < 17.1 cm; mean ± SD: 13.63 ± 1.20 cm) and weight composition (♂: 4.95 < W < 28.25 g; mean ± SD: 15.40 ± 4.61g; ♀: 4.91 < W < 34.29 g; mean ± SD: 15.56 ± 5.12 g).

Diet composition

Out of 295 anchovy specimens examined, 270 (91.5%) had only animal food in their stomachs, among which, 13 taxonomic groups and 12,234 prey items were identified (Table 2). The abundance and frequency of occurrence of all the prey categories are reported in Table 1 and according to presented data, copepods and decapods were consistently the most frequent and abundant prey items in anchovy in the Adriatic Sea.

Although a low linear correlation in the relationship of %Jr and TL was observed (%Jr = 3.89 - 0.22*TL, r = -0.245; Fig. 1), the vacuity index was larger in medium- than in smaller-sized anchovy (10.64% as opposed

Table 1. Descriptive overview of seasonal sardine and anchovy samples (N - number of individuals, TL - total length in cm, W - total body weight in g, Wsf - weight of full stomach in g) collected with commercial purse seiners in the eastern Adriatic Sea through the investigated period (January-December, 2011).

Season		N	range TL (cm; mean TL±SD)	range W (g; mean W±SD)	range W_{sf} (g; mean W_{sf}±SD)
			***Sardina pilchardus***		
Winter	♂	34	12.2 - 17.1 (14.26±1.39)	12.09 - 36.12 (20.66±6.65)	0.13 - 0.53 (0.28±0.12)
	♀	34	12.5 - 19.3 (14.76±1.49)	14.46 - 50.01 (23.60±8.60)	0.10 - 1.12 (0.36±0.24)
	Total	68	12.2 - 19.3 (14.51±1.46)	12.09 - 50.01 (22.13±7.77)	0.10 - 1.12 (0.32±0.20)
Spring	♂	54	12.2 - 17.8 (14.64±1.37)	12.94 - 39.21 (22.63±6.93)	0.10 - 0.83 (0.32±0.17)
	♀	31	12.7 - 17.7 (15.54±1.41)	14.93 - 38.18 (26.22±7.31)	0.14 - 0.90 (0.37±0.19)
	Total	85	12.2 - 17.8 (14.97±1.44)	12.94 - 39.21 (2394±7.24)	0.10 - 0.90 (0.34±0.18)
Summer	♂	34	13.4 -19.7 (15.83±1.05)	18.72 - 54.27 (30.99±6.21)	0.24 - 0.82 (0.41±0.14)
	♀	13	14.1 - 18.7 (16.38±1.31)	21.05 - 50.07 (34.76±8.15)	0.29 - 0.86 (0.49±0.15)
	Total	54	13.4 - 19.7 (15.90±1.11)	18.72 - 54.27 (31.54±6.67)	0.20 - 0.86 (0.41±0.15)
Autmun	♂	20	14.2 - 16.9 (15.36±0.78)	19.99 - 35.88 (27.24±4.09)	0.34 - 0.86 (0.54±0.16)
	♀	17	14.5 - 16.5 (15.57±0.49)	21.59 - 32.75 (28.89±2.99)	0.40 - 0.92 (0.65±0.15)
	Total	37	14.2 - 16.9 (15.45±0.67)	19.99 - 35.88 (27.98±3.69)	0.34 - 0.92 (0.59±0.16)
			***Engraulis encrasicolus***		
Winter	♂	32	9.9 - 14.5 (12.57±1.13)	4.95 - 15.18 (11.20±2.86)	0.06 - 1.01 (0.28±0.23)
	♀	48	9.9 - 14.4 (12.71±1.10)	4.91 - 16.56 (11.71±2.90)	0.04 - 0.52 (0.22±0.12)
	Total	89	9.9 - 14.5 (12.60±1.15)	4.91 - 16.56 (11.35±3.01)	0.04 - 1.01 (0.24±0.18)
Spring	♂	47	12.1 - 16.1 (14.12±1.17)	9.49 - 28.25 (17.46±4.94)	0.11 - 1.05 (0.33±0.22)
	♀	40	11.9 - 17.0 (14.11±1.22)	10.55 - 34.29 (17.37±5.52)	0.13 - 1.04 (0.34±0.23)
	Total	87	11.9 - 17.0 (14.11±1.19)	9.49 - 34.29 (17.42±5.18)	0.11 - 1.05 (0.34±0.23)
Summer	♂	37	12.2 - 16.8 (13.99±0.90)	10.33 - 27.84 (16.89±3.64)	0.11 - 0.31 (0.19±0.05)
	♀	50	11.7 - 17.1 (14.18±1.16)	9.29 - 31.51 (18.13±4.92)	0.10 - 0.38 (0.21±0.07)
	Total	89	11.7 - 17.1 (14.09±1.05)	9.29 - 31.51 (17.57±4.40)	0.10 - 0.38 (0.20±0.06)
Autmun	♂	15	12.7 - 14.6 (13.45±0.49)	12.24 - 18.32 (14.26±1.63)	0.20 - 0.59 (0.33±0.10)
	♀	15	12.4 - 14.3 (13.41±0.44)	12.48 - 17.46 (14.46±1.46)	0.19 - 0.74 (0.38±0.14)
	Total	30	12.4 - 14.6 (13.42±0.46)	12.24 - 18.32 (14.38±1.55)	0.19 - 0.74 (0.36±0.12)

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Table 2. Frequency of occurrence (%F) and abundance (%N) of prey items found in the anchovy (A) and sardine (S) stomach collected in the eastern Adriatic Sea during different seasons and over the whole investigated period (January-December, 2011).

Prey item	Total Sardine		Total Anchovy		Winter Sardine		Winter Anchovy		Spring Sardine		Spring Anchovy		Summer Sardine		Summer Anchovy		Autumn Sardine		Autumn Anchovy	
	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N
Copepoda	86.5	58.3	85.4	56.2	88.2	86.9	98.9	78.6	83.5	76.4	73.6	37.6	85.2	56.1	79.8	72.0	92.1	31.7	96.7	19.8
Amphipoda	1.6	0.2	7.1	0.5	2.9	0.3	2.2	<0.1	3.5	0.3	16.1	2.9	7.4	1.4	3.4	0.5	0.0	0.0	6.7	0.1
Mysidacea	2.0	0.4	15.6	1.5	17.7	1.2	24.7	2.1	4.7	0.2	16.1	2.4	1.9	0.7	4.5	0.5	10.5	0.1	20.0	0.2
Euphausiacea	0.8	4.8	10.2	0.5	13.2	1.3	19.1	0.6	11.8	15.9	5.7	0.4	1.9	0.3	5.6	0.9	2.6	<0.1	10.0	0.2
Cladocera	1.2	<0.1	0.3	<0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.6	1.0	1.1	0.1	0.0	0.0	0.0	0.0
Cirripedia	3.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.8	3.4	0.0	0.0	0.0	0.0	0.0	0.0
Decapoda larvae	17.1	33.2	56.9	36.1	50.0	5.7	62.9	15.7	42.4	5.9	48.3	42.7	29.6	7.1	44.9	9.1	81.6	67.4	100.0	79.1
Pisces ova	6.9	1.7	28.8	3.1	29.4	3.2	37.1	0.7	12.9	0.4	27.6	11.9	25.9	23.3	31.5	11.3	15.8	0.2	0.0	0.0
Pisces larvae	0.0	0.0	8.1	0.5	0.0	0.0	12.4	0.5	0.0	0.0	13.8	1.4	0.0	0.0	0.0	0.0	0.0	0.0	3.3	<0.1
Bivalvia	4.1	0.4	19.7	1.2	1.5	<0.1	34.8	1.7	10.6	0.6	6.9	0.3	18.5	4.4	16.9	2.5	7.9	0.1	20.0	0.2
Gastropoda	0.8	0.1	3.1	0.1	2.9	<0.1	0.0	0.0	1.2	0.1	0.0	0.0	3.7	0.7	9.0	1.3	2.6	<0.1	3.3	<0.1
Echinodermata	0.0	<0.1	0.7	0.1	1.5	<0.1	0.0	0.0	2.4	0.1	0.0	0.0	0.0	0.0	2.2	0.9	0.0	0.0	0.0	0.0
Ostracoda	0.0	<0.1	1.4	<0.1	1.5	0.1	0.0	0.0	0.0	0.0	2.3	0.1	0.0	0.0	2.2	0.2	0.0	0.0	0.0	0.0
Polichaeta	1.2	0.1	5.1	0.2	0.0	0.0	0.0	0.0	2.4	0.1	4.6	0.2	7.4	1.7	6.7	0.7	0.0	0.0	16.7	0.2
Cephalochordata	0.0	<0.1	0.0	0.0	0.0	0.0	0.0	0.0	1.2	<0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Diatomeae	3.7	0.2	0.0	0.0	1.5	0.2	0.0	0.0	1.2	<0.1	0.0	0.0	0.0	0.0	0.0	0.0	23.7	0.4	0.0	0.0
Dinophyceae	0.4	0.3	0.0	0.0	14.7	1.0	0.0	0.0	1.2	<0.1	0.0	0.0	0.0	0.0	0.0	0.0	2.6	<0.1	0.0	0.0

to 2.53%); small- (TL < 12.9 cm) and medium-sized (TL > 13.0 cm) anchovy generally showed no significant differences in diet composition (ANOSIM test, $r = -0.045$, $\alpha = 0.965$, $p > 0.05$). However, copepods were the most frequent prey in both categories, but in larger specimens, larvae of decapods were more abundant than of copepods.

From a total of 131 male anchovy, 10.69% had an empty stomach, whereas 6.54% of 153 female anchovy had no prey items in the stomach. Nevertheless, no significant difference in the diet between sexes was observed
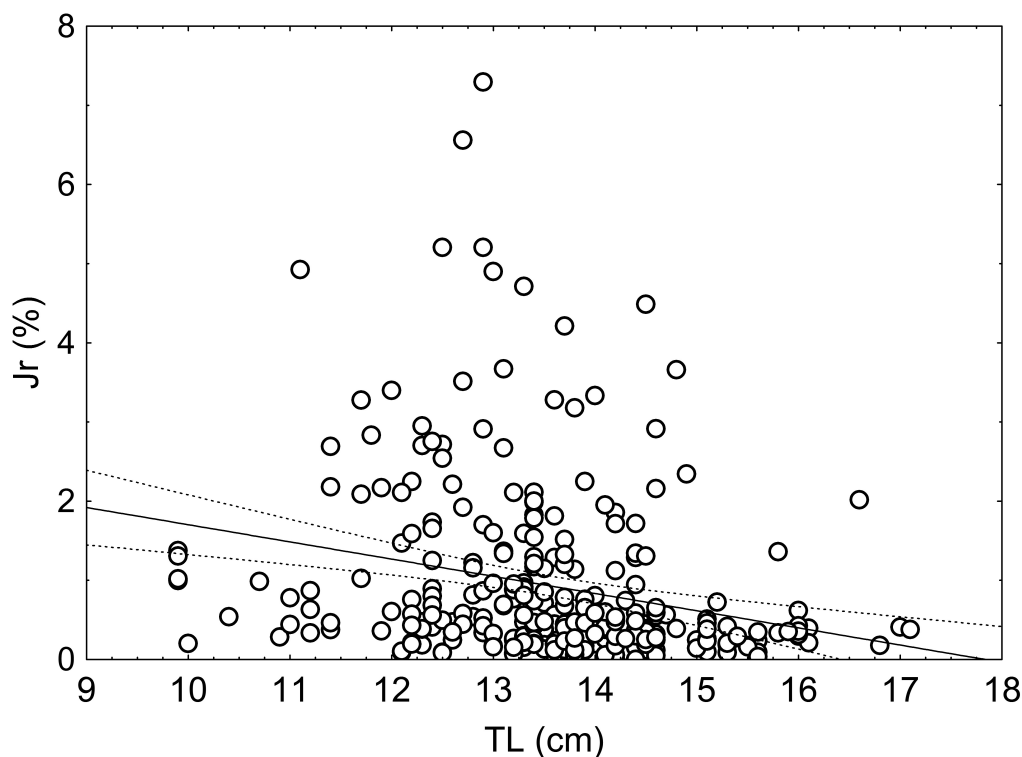

Fig. 1: Oscillations in the fullness index (%Jr,) with size (TL, cm) of anchovy specimens, eastern Adriatic Sea, January-December 2011.

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(ANOSIM test, $r = -0.012$, $\alpha = 0.066$, $p > 0.05$), since copepods were the most frequent and abundant prey in the stomachs of both sexes.

Analysing seasonal changes, we observed that the number of empty stomachs as well as their fullness varied; during the winter and autumn, more individuals (3.37 and 6.67, respectively), had prey in their stomach than during the spring and summer (11.49 and 11.24, respectively). The %Jr ranged from 1.26 (winter) to 0.30 (autumn). Although copepods were the most frequent prey item in all seasons, their abundance was dominant in the winter and summer period, whereas in the autumn and spring, the most abundant prey type were decapod larvae (Table 2). The ANOSIM test showed a significant statistical difference in prey composition between seasons (global $r = 0.114$, $\alpha = 0.01$, $p < 0.05$) and the most pronounced difference in anchovy diet was observed in the summer–autumn season, whereas no statistically significant difference was found in the spring–autumn period (Table 3).

Population dynamic of Parahemiurus merus and Hysterothylacium aduncum in anchovy

The one-year prevalence of *P. merus* in the anchovy population (N = 295) was 21.7% (95% confidence limit 17.12–26.85); the intensity was 2.84 (bootstrap 95% confidence limit 2.23–3.61); the abundance was 0.62 (bootstrap 95% confidence limit 0.43–0.82) and the mean crowding was 5.58 (bootstrap 95% confidence limit 4.61–6.66). The variance-to-mean ratio was 4.982 and the index of discrepancy was 0.882. After fitting the negative binomial distribution as a theoretical model to the observed data following the maximum-likelihood method, the observed and expected frequencies of *P. merus* did not differ significantly ($p = 0.05$, exponent of the negative binomial k = 0.145), demonstrating its aggregation across host populations (left-biased). The smallest infected anchovy was 10.7 cm long and 7.1 g in weight (female, winter), whereas the largest measured 16.8 cm and 27.84 g (male, summer). The greatest number of parasites (N = 11) were found in a female of 16 cm and 23.69 g in summer. Comparing the one-year prevalence of *P. merus*, abundance and intensity values between anchovy and sardine, a difference was observed

for the prevalence, abundance and mean crowding, but not for the mean intensity ($p < 0.05$).

The consensus tree inferred from NJ analysis showed phylogenetic relationships for anisakids (Fig. 3), confirming the morphological identification of *H. aduncum* larvae. Because all larvae isolated from Adriatic sardine and anchovy conformed to the same haplotype, we have depicted them as a single isolate (sardine/anchovy isolate, Fig. 3), which forms a sister clade to other two isolates of Adriatic *H. aduncum*. The one-year prevalence of *H. aduncum* in the anchovy population (N = 295) was 10.5% (95% confidence limits 7.25–14.59); the intensity was 1.42 (bootstrap 95% confidence limits 1.23–1.58); the abundance was 0.15 (bootstrap 95% confidence limits 0.1-0.2) and mean crowding was 1.59 (bootstrap 95% confidence limits 1.37–1.71). The variance-to-mean ratio was 1.45 and the index of discrepancy was 0.91. The negative binomial distribution of this ascarid as a fit to the theoretical model could not be tested because only two abundance classes existed. The smallest anchovy infected by *H. aduncum* measured 11.1 cm and 6.9 g (female, winter), whereas the largest measured 16.8 cm and 27.84 g (male, summer). The greatest number of parasites (N = 2) was found in a few anchovies throughout all seasons of the year sampled. Comparing the one-year *H. aduncum* prevalence, abundance and intensity values between anchovy and sardine, no difference was observed for prevalence, abundance or mean intensity ($p < 0.05$). Similarly, the intensity distribution and mean crowding of *H. aduncum* were not statistically different. Seasonal variations in the measured population dynamic parameters of the two parasitic species are given in Table 5.

Sardine

Length-frequency distribution

During the one-year research period (January–December, 2011), all collected and analysed sardine specimens varied in total length from 7.0 to 21.0 cm (mean ± SD: 13.98 ± 0.78 cm). The length-frequency distribution of sardine subsamples collected for stomach content analysis did not differ significantly (Wald–Wolfowitz Runs Test; sardine: $Z = 0.418$, $p = 0.676$) from that obtained in purse-seine catches throughout the investigated

Table 3. Results of the one-way and two-way ANOSIM test of seasonal differences in anchovy and sardine as well as among two of them.

Season	Anchovy		Sardine		Anchovy *vs* Sardine	
	R	P	R	P	R	P
Winter versus Spring	0.082	0.001	0.016	0.096	0.052	0.001
Winter versus Summer	0.135	0.001	0.070	0.005	0.113	0.001
Winter versus Autumn	0.134	0.006	0.217	0.001	0.168	0.001
Spring versus Summer	0.067	0.001	0.090	0.006	0.076	0.001
Spring versus Autumn	0.000	0.456	0.172	0.001	0.086	0.004
Summer versus Autumn	0.331	0.001	0.499	0.001	0.378	0.001

Table 4. Results of a SIMPER test on seasonal differences between the anchovy and sardine diet.

Variable	First group average abundance	Second group average abundance	Average dissimilarity	SD	% contribution	Cumulative %
Winter versus Spring						
Copepoda	2.72	1.94	21.43	1.41	37.96	37.96
Larvae decapoda	1.13	0.93	13.22	1.15	23.42	61.38
Eggs	0.36	0.29	6.86	0.61	12.15	73.53
Winter versus Summer						
Copepoda	2.72	1.58	21.29	1.46	37.57	37.57
Larvae decapoda	1.13	0.43	12.35	1.25	21.80	59.38
Eggs	0.36	0.46	7.63	0.71	13.47	72.85
Winter versus Autumn						
Larvae decapoda	1.13	3.88	25.79	1.68	48.14	48.14
Copepoda	2.72	3.02	14.68	1.40	27.41	75.54
Eggs	0.36	0.08	3.25	0.57	6.07	81.62
Spring versus Summer						
Copepoda	1.94	1.58	20.83	1.28	34.92	34.92
Larvae decapoda	0.93	0.43	13.44	1.01	22.53	57.44
Eggs	0.29	0.46	9.76	0.69	16.36	73.80
Spring versus Autumn						
Larvae decapoda	0.93	3.88	28.59	1.91	50.05	50.05
Copepoda	1.94	3.02	16.10	1.34	28.19	78.25
Eggs	0.29	0.08	3.14	0.48	5.50	83.75
Summer versus Autumn						
Larvae decapoda	0.43	3.88	34.18	2.76	52.34	52.34
Copepoda	1.58	3.02	17.25	1.66	26.42	78.76
Eggs	0.46	0.08	4.40	0.60	6.73	85.49

year. The total length of sardine specimens collected for stomach content analysis ranged from 12.2 to 19.7 cm (N = 245; mean ± SD: 15.12 ± 1.38 cm), whereas their total body weight was between 12.09 and 54.27 g (mean ± SD: 25.74 ± 7.70 g). In the subsample, the total length and weight range of male and female sardines was almost the same (♂: N = 93; 12.5 < TL < 19.3 cm; mean TL ± SD: 15.42 ± 1.38 cm; 12.09 < W < 54.27 g; mean W ± SD: 24.83 ± 7.45 g; ♀: N=145; 12.2 < TL < 19.7 cm; mean ± SD: 14.92 ± 1.36 cm; 14.46 < W < 50.07 g; mean W ± SD: 26.93 ± 8.14 g). Although the overall sex ratio (m/f = 0.64) indicated that females statistically dominated over males ($\chi^2 = 11.361$, df = 1, $p < 0.001$), no difference was detected between the length distribution of male and female sardine specimens (Kolmogorov-Smirnov Test, $p < 0.05$).

Diet composition

Out of 245 sardine specimens examined, 226 (92.2%) had food in their stomachs. The analysis of stomach contents indicated that sardines preferred to feed on animal food (91.2%), although a plant food component was also present. In sardine stomachs, a total of 9,017 prey items belonging to 17 taxonomic groups were identified and their abundance and frequency of occurrence are reported in Table 2. Accordingly, copepods and decapods were

consistently the most frequent and abundant prey items in the sardine diet (Table 2).

With respect to three sardine length groups, prey composition did not differ significantly (ANOSIM, Global R = -0.015; α = 0.795; $p > 0.05$). The diet composition of sardine was not related to size, as all examined sardines preyed mainly upon copepods, larval decapods and eggs. The proportion of empty guts varied from 0.03% (TL=37.0 cm, W = 52.78 g) to 2.94% (TL = 37.5 cm, W = 66.83 g). Although a slight increase in the fullness index with sardine size was observed (%Jr = 0.069 + 0.033TL; Fig. 2), it was not statistically significant ($r = 0.087$, $p = 0.180$). Congruent with low value of fullness index (mean ± SD: 0.57 ± 0.52%), a low vacuity index was also observed (%V = 7.76 %).

In terms of the relationship between diet composition and sardine sex, no statistically significant difference (ANOSIM, Global R = 0.015; α = 0.165; $p > 0.05$) was found. Once again, copepods and decapods larvae were confirmed as most-preferred prey items.

Feeding activity varied over the year; the percentage of empty stomachs or the vacuity index (%V) was highest in spring (%V = 32.94%) and lowest in winter (%V = 5.88%).

In terms of seasonal diet composition (Table 2), copepod and decapod larvae as the second most impor-

Table 5. Values of prevalence (%) with confidence intervals (CI), mean intensity with confidence intervals (CI), and mean abundance with confidence intervals (CI) of two parasites: *Parahemiurus merus* (Digenea) and *Hysterothylacium aduncum* sampled seasonally from the anchovy (*Engrauils encrasicolus*) and sardine (*Sardina pilchardus*).

Host	N	Parasite	Season	Prevalence (%) (CI)	Intensity (CI)	Abundance (CI)	k
anchovy (N=295)	89	*Parahemiurus merus*	winter	14.6 (8-23.69)	1.31 (1-1.69)	0.19 (0.11-0.29)	/
	87		spring	4.6 (1.32-11.36)	1 (/)	0.05 (0.01-0.09)	/
	89		summer	49.4 (38.66-60.25)	3.59 (2.7-4.5)	1.78 (1.27-2.46)	0.392
	30		autumn	10 (2.11-26.53)	1 (/)	0.1 (0-0.2)	/
	89	*Hysterothylacium aduncum*	winter	4.5 (1.23-11.11)	1.25 (1-1.5)	0.06 (0.01-0.12)	/
	87		spring	5.7 (1.89-12.91)	1.4 (1-1.6)	0.08 (0.02-0.17)	/
	89		summer	11.2 (6.79-19.54)	1.1 (1-1.3)	0.12 (0.06-0.2)	/
	30		autumn	10 (2.11-26.53)	1 (/)	0.1 (0-0.2)	/
sardine (N=245)	68	*Parahemiurus merus*	winter	52.9 (40.44-65.17)	2.22 (1.75-2.89)	1.18 (0.81-1.62)	0.807
	85		spring	42.4 (31.7-53.56)	2.08 (1.69-2.72)	0.88 (0.61-1.2)	0.635*
	54		summer	46.3 (32.62-60.4)	2.4 (1.88-3.08)	1.11 (0.74-1.54)	0.657*
	38		autumn	28.9 (15.42-45.91)	2.73 (1.91-3.64)	0.79 (0.39-1.34)	0.252*
	68	*Hysterothylacium aduncum*	winter	10.3 (4.24-20.07)	1.29 (1-1.57)	0.13 (0.04-0.24)	/
	85		spring	7.1 (2.63-14.74)	1.17 (1-1.5)	0.08 (0.02-0.16)	/
	54		summer	16.7 (7.91-29.3)	2 (1.22-3.33)	0.33 (0.15-0.7)	0.165*
	38		autumn	10.5 (2.94-24.81)	2 (1-3.25)	0.21 (0.05-0.55)	/

* the negative binomial k represents the observed frequencies of parasites that do not statistically differ from theoretical model.

tant group were the most frequent prey items in sardine stomachs throughout the year. The seasonal pattern of prey item abundance mainly confirmed copepod and decapod larvae as the preferred sardine food, except in spring and summer, when the second food category comprised euphausidae and fish eggs, respectively. Although no seasonality in the sardine diet was observed, statistical analysis highlights that the feed significantly differs (ANOSIM, Global $r = 0.133$; $\alpha = 0.01$; $p < 0.05$) throughout the year. The most pronounced difference in sardine diet composition was observed between summer and autumn (Table 3), as the abundance index of copepods and eggs was highest in summer, whereas in autumn, the same index distinguished decapod and copepod larvae as being most abundant (Table 2). The ANOSIM test showed significant similarity only between the winter and spring periods (Table 3); copepods were the most frequent and abundant prey group (Table 2).

Population dynamic of P. merus and H. aduncum in sardine

The one-year prevalence of *P. merus* in the sardine population (N = 245) was 44.1% (95% confidence limit 37.76–50.55); the intensity was 2.27 (bootstrap 95% confidence limits 1.98–2.59); the abundance was 1.00 (bootstrap 95% confidence limits 0.82–1.18) and the mean crowding was 3.42 (bootstrap 95% confidence limit 2.96–4.03). The variance-to-mean ratio was 2.43 and the index of discrepancy, 0.72. After fitting the negative binomial distribution as a theoretical model to the observed data following the maximum-likelihood method, the observed and expected frequencies of *P. merus* did not differ significantly ($p = 0.05$; the exponent of the negative binomial k = 0.60), demonstrating its aggregation across host populations (left-biased). The smallest infected sardine measured 12.5 cm and 14.46 g (female, winter),
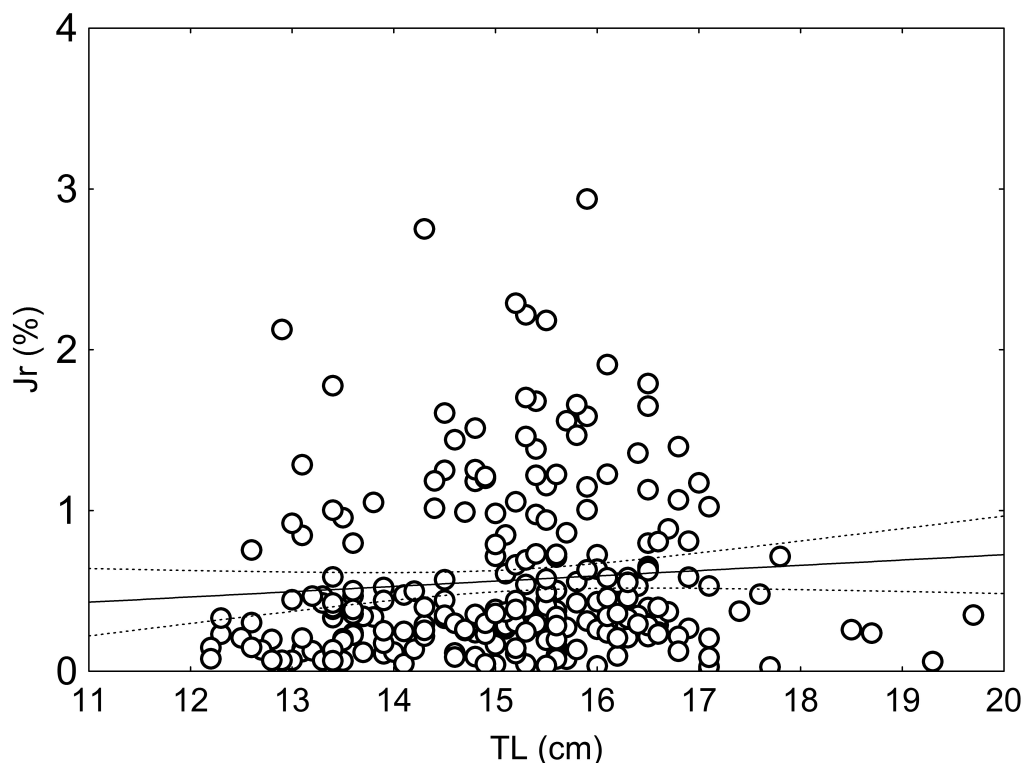
Fig. 2: Oscillations in the fullness index (%Jr,) with size (TL, cm) of sardine specimens, eastern Adriatic Sea, January-December 2011.

whereas the largest measured 18.5 cm and 42.54 g (female, summer). The greatest number of parasites (N = 8) was found in a female of 17.7 cm and 38.18 g in spring.

The one-year prevalence of *H. aduncum* in the sardine population (N = 245) was 10.6% (95% confidence limits 7.05–15.17); the intensity was 1.62 (bootstrap 95% confidence limits 1.27–2.27); the abundance was 0.17 (bootstrap 95% confidence limits 0.11–0.27) and the mean crowding was 2.43 (bootstrap 95% confidence limits 1.63–3.89). The variance-to-mean ratio was 2.27 and the index of discrepancy was 0.92. After fitting the negative binomial distribution as a theoretical model to the observed data following the maximum-likelihood method, the observed and expected frequencies of this anisakid did not differ significantly ($p = 0.05$; the exponent of the negative binomial k = 0.14), demonstrating its aggregation across host populations (left-biased). The smallest infected sardine measured 12.2 cm and 13.57 g (male, winter) and the largest measured 19.7 cm and 54.27 g (male, summer). The greatest number of parasites (N = 6) was found in a female of 17.1 cm and 45.25 g in summer. The seasonal variations in the measured population dynamic parameters of both parasitic species are given in Table 5.

Comparison of the diet of anchovy and sardine

Anchovy and sardine appeared to feed quite similarly, as their dietary diversity was 1.48 and 1.50, respectively.

Equitability was also slightly higher in anchovy ($E_A = 0.40$) than in sardine ($E_S = 0.37$), generally emphasising that in each species, each prey was not represented in the same percentage by number. The diet overlap calculated by the Schoener's index was 0.93 by relative abundance, which confirmed the almost complete overlap in the diet of these two highly important pelagic fish species.

Two-way ANOSIM was used to test the observed differences in diet between sardine and anchovy in relation to season. The results showed that the diets of both species differed in all seasons (Table 3). Differences were less pronounced between winter and spring, whereas they were greatest in the summer–autumn period. Applying SIMPER to assess the relative contributions of each prey group to the dissimilarities/similarities among two pelagic fish species, copepod and decapod larvae and fish eggs mostly contributed to the dissimilarity in feeding over the investigated period (Table 4); decapod larvae in winter–autumn, spring–autumn and summer–autumn and copepods in the winter–spring, winter–summer, spring–summer period.

Discussion

Each marine ecosystem is complex with respect to its species composition and to the interactions between them. Species interactions are important factors that influence community structure and the relative abundance of species, including inter- and intra- specific predation
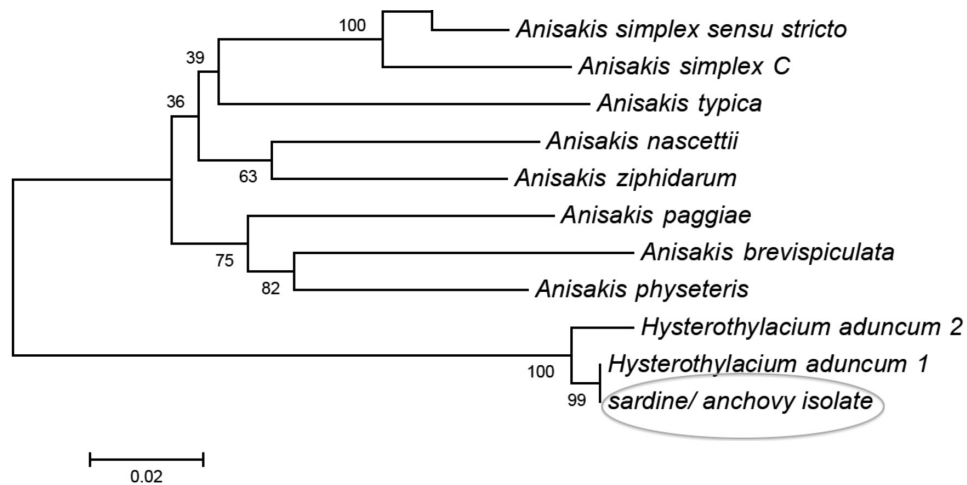
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Fig. 3: Unrooted consensus tree inferred by Neighbour-Joining analysis, showing phylogenetic relationships for selected anisakid sequences. *Hysterothylacium aduncum* larvae isolated in this study are depicted as a single isolate (sardine/anchovy isolate, encircled).

and competition (Wootton, 1994). Species compete for food and/or space when they share similar resources or similar ecological characteristics and these can change through time and space (Huston, 1979). Nevertheless, the dietary composition of planktivorous species might at least partly reflect the biomass and distribution of phytoplankton and zooplankton. Knowledge on the diet composition of small pelagic fish species such as anchovy and sardine, which are important prey for carnivorous predators and important predators on plankton, could provide, useful information as a possible indicator concerning the structure of the food web and ecosystem.

In the present study, 295 anchovy specimens (9.9 < TL < 17.1 cm; mean ± SD: 13.58 ± 1.27 cm) and 245 sardine specimens (7.0 < TL < 21.0 cm; mean ± SD: 13.98 ± 0.78 cm) collected from commercial purse-seine catches were collected from the eastern part of the Adriatic Sea between January 2011 and December 2011 and were analysed to determine their feeding habits. The scarcity of young anchovy and sardine specimens in length frequency distribution in subsamples collected for stomach content analysis was because in the Mediterranean, including the Adriatic, the minimum legal landing sizes for anchovy and sardine are 9 cm and 11 cm (Council Regulation (EC) No. 1967/2006), respectively. Nevertheless, the observed length ranges of both species were in compliance with previous studies on this species and with this fishing technique (Sinovčić *et al*., 2004; Sinovčić & Zorica, 2006; Zorica *et al*., 2007; Sinovčić *et al*., 2008).

The overall stomach content analysis indicated that sardines and anchovy generally preferred to feed on animal food, although in sardines, a plant component (4.1%) was also present. The diets of sardine and anchovy were broadly similar and in both species were dominated by copepods and decapod larvae, although in different proportions (Table 2). Since copepods are on average the most abundant group in mesozooplankton and generally

correspond to the size fraction of the preferred prey range of the sardine and anchovy (Plounevez & Champalbert, 1996; Garrido *et al*., 2007), their dominance in the respective fish diets might partly be a reflection of their vast availability. A steady animal food supply is also provided by the miscellaneous decapod larvae, which are an important part of meroplankton. A high abundance of this prey type is ensured through high diversity (Lučić, 1998) and life strategies that include multiple spawning and multiple moults, resulting in the protracted life cycle of many species. The variable degree of exploitation of other mesozooplankton groups could be related to some restrictive aspects of their distribution (e.g. nearshore abundance and temperature-related seasonality of cladocerans and cirriped larvae), or to lower-chance encounters with prey that is characterised by high patchiness (e.g. mysids, euphausiids, amphipods). The preference of decapod larvae over copepod prey in larger anchovies is size-related, since copepods are gradually substituted by larger crustaceans such as decapods and amphipods in the diet of age groups 1, 2 and 3 (Bacha & Amara, 2009).

Furthermore, as previously stated, the obvious difference among investigated species was related to diatoms and dinoflagellates that are noticeably consumed only by sardines. The general dietary regimes for adult specimens of both species agreed with findings of previous studies. The majority of studies confirmed anchovy to be exclusively zooplanktivorous (Tudela & Palomera, 1997; Coombs *et al*., 1997; Conway *et al*., 1998; Borme *et al*., 2009), except a few studies in which phytoplankton was also present. For instance, Karlovac (1967) and Regner (1985) reported the presence of phytoplankton in the guts of anchovy larval stages in the Adriatic Sea, whereas sporadic feeding of adult anchovies on phytoplankton in other geographical areas was recorded by Mikhman & Tomanovich (1977), James & Findlay (1989), Bul-

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gakova (1993) and Budnichenko *et al*. (1999). Many authors have reported that adult sardines feed on both, zooplankton (mainly adult and juvenile copepods) and phytoplankton, but whereas some support the thesis of phytoplankton dominance in stomach contents (Cépède, 1907; Oliver, 1951; Oliver & Navarro, 1952), others have found the opposite (Varela *et al*., 1990; Bode *et al*., 2003; Cunha *et al*., 2005). Observed differences in feeding habits within the same species are probably attributable to differences in prey availability as a response to the existence of differing hydrographic conditions among the investigated areas.

For both species, sex or length classes did not influence diet composition, therefore, no significant size-related diet shift in either investigated pelagic fish species was observed. The obtained results confirmed the hypothesis of Costalago *et al*. (2012) that the diet shift in sardine occurs primarily at the time of metamorphosis, whereas juveniles and adults maintain fairly similar diets. Ontogenetic diet shifts have been usually related to larval diet composition, and are strongly correlated to the development of feeding apparatus, visual acuity and swimming ability (Dulčić, 1999; Morote *et al*., 2010; Borme *et al*., 2013). With respect to putative sex differences, male and female diet composition did not vary in anchovies and sardines. Tudela & Palomera (1997) previously investigated some aspects of anchovy diet related to sex, and found that diet remained the same between sexes throughout the year. Feeding habits were not influenced by spawning season, congruent with the findings in this study.

The diet composition of two pelagic fish showed significant seasonal variation. The results highlighted that anchovies tend to feed on larger and therefore higher energy-content prey (decapod larvae) during spring and autumn, whereas sardines feed on these type of prey (euphausids and fish eggs) only in spring and summer. This might merely reflect changes in the abundance and availability of various prey species, although more likely it relates to species physiology. Spawning activity, which in the Adriatic anchovy occurs between April and September (spring–summer) and in sardine from October until April (autumn–winter), coincides with higher energy-content prey, which supports the elevated requirements of fish to accumulate and/or renew the energy lost due to spawning.

Gastrointestinal helminths also reflect differences in anchovy and sardine feeding behaviour. Although the overall prevalence of *H. aduncum* in sardine and anchovy is the same, differences in digenean prevalence between the two hosts are almost double. *Parahemiurus merus* is a widely dispersed hemiurid digenean, whose life cycle is practically unknown. It usually infects temperate pelagic fishes (e.g. clupeids, carangids, salmonids and engraulids) and chaetognaths from most oceans (Bray, 1990). Almeida *et al*. (2009) identified progenetic metacercariae of *P. merus* in the chaetognath *Parasagitta*

friderici (Ritter-Zahony, 1911), suggesting that its infection might occur through predation on infected copepods or cladocerans, since it preys on zooplankton (Dollfus, 1960; Pearre, 1976). However, the authors did not isolate metacercariae from the digestive tract lumen, but from the body cavity of the worm, indicating that the real role of chaetognaths in the *P. merus* life cycle is still uncertain. Unfortunately, little is known concerning the general host occurrence of *P. merus* in the Adriatic. Paradižnik & Radujković (2007) did not list it when assessing digenean trematodes in the north Adriatic in the same hosts, potentially because of discrepancies between their and our study's geographic area and its environmental profile, influencing parasite communities. In contrast to *P. merus*, the life cycle and host range of *H. aduncum* have been more elucidated, but its highly generalist nature that enables the dissemination of II stage larvae through many different organisms (barnacles, calanoid copepods, polychaets, gammarid amphipods) (Gonzalez & Carvajal, 1995), prevents pinpointing its source in sardine and anchovy in the Adriatic. Whether an unknown intermediate host of *P. merus* is the preferred prey in sardine, the ubiquitous character of *H. aduncum* II stage larvae enables equal parasite population parameters in both sardine and anchovy. Furthermore, trophic relationships vary over time and space, and animals with size- and age-related diet changes often alter the parasites that they harbour, indicating ontogenic shifts in feeding behavior (Marcogliese, 2005). Evidence from the helminth community of the Adriatic sardine and anchovy populations indicates the existence of early ontogenic shift (from larvae to juveniles/adults) in their feeding behavior, probably due to morphological changes that might encourage aggregation of the digeanean, in contrast to an ubiquitous nematode. Consequently, helminth community in studied hosts is not reflection of habitat change, given that both species are sympatric in the Adriatic. The consistent presence of only two helminth taxa in both species indicates decisive feeding patterns, coevolved predator–prey relationships, and stability within the ecosystem (Marcogliese, 2005).

Finally, this study confirmed the great similarity that was expected between the sardine and anchovy diet, since they inhabit the same ecological niche. This observed overlap is of great importance for analysis of the population dynamics of these species and should be considered and implemented in more suitable ecosystem models in future. Despite dietary overlap, the sardine and anchovy stomach content analysis suggested that their feeding habits varied seasonally in prey species quantitatively, not qualitatively. Nevertheless, future studies should analyse the stomach contents of those two very important small pelagic fish species to lower taxonomic levels and/or use stable isotope analysis, to define possible difference as well as to confirm direct links between nutrients and the evident oscillation in sardine and anchovy stock biomass that affect species at higher trophic levels.

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Acknowledgements

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