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Ascaridoid parasites in European sardine throughout the annual cycle: Variability in parasitic load according to host stock features

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ABSTRACT

In recent years, a drop in the condition of the European sardine has been observed. Although several causes have been attributed to this issue, as overfishing and climate change, little is known about the link between ascaridoid nematode parasitisation and fish status. In this study, sardines were obtained from four fishing grounds along the Mediterranean (Alboran, Northern Spain, Northern Adriatic, and Aegean), and one location in the Atlantic Ocean (Southern Portugal). After analysing individual fish body condition (by direct tissue fat content measurements and condition indices), and reproductive status (by a detailed gonadal examination) throughout the entire annual cycle, ascaridoids were recognised by combining naked eye and UV-press method along flesh, viscera, and gonads. Afterwards, sequence analysis of the rDNA internal transcribed spacers region (ITS) and the mtDNA *cox2* gene were used to identify and characterise the different species of ascaridoids from the fish host in the localities throughout the seasons. The main species found along different areas was *Hysterothylacium aduncum*, present in the Northern Adriatic (prevalence of 7.6%, mean intensity 1.700), the Atlantic (7.5%, 3.889), and the Northern Spain (3.9%, 1.600). Moreover, few individuals of *Anisakis simplex* (s.s.) and *A. pegreffii* were observed in the Atlantic (1.7% and 0.8%, respectively), and the latter species was also found in the Adriatic stock (0.8%). All ascaridoid specimens were found in viscera. Obtained results seem to indicate that in stocks with medium sizes, small variations in length are related to parasite intensity. This study highlights the importance of seasonal parasitological analyses at stock level and, especially, in capital breeders, as relationships between condition and reproduction parameters and parasitism are conditioned by seasonality.

1. Introduction

European sardine (*Sardina pilchardus* (Walbaum, 1792)) is a small pelagic fish from the Clupeidae family with cold-temperate water affinity, living within a depth range of 10–100 m (Renzi et al., 2019). It plays an important role in the ecosystem as a filter feeder (Cury et al., 2000; Van Beveren et al., 2014) that feeds mainly on planktonic crustaceans, appendicularians, diatoms and other organisms (Costalago and Palomera, 2014), contributing to the energy transfer to higher trophic levels. From the point of view of human nutrition, sardine is rich in long-chain polyunsaturated fatty acids (PUFA), essential for human development and the prevention of many health disorders, as well as easy digestible proteins which contain all essential amino acids necessary for healthy human diets, minerals, and vitamins (Šimat et al., 2020).

It is found throughout the northeast of the Atlantic Ocean, from the North Sea to Mauritania and Senegal, the Sea of Marmara, the Black Sea, and the Mediterranean Sea (Parrish et al., 1989). Specifically, in the latter most stocks have exhibited declining trends in terms of abundance (Tugores et al., 2011; FAO, 2020; Fernandes et al., 2017), remaining low in the present day (FAO, 2020). In addition to the problems that could be reflected in the food chain due to its relevance as a foraging species, the economic consequences are obvious since together with anchovy, sardine traditionally provides the largest catches in this area (Lleonart and Maynou, 2003).

This decline in production is linked to a decrease in somatic

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condition, length at age, and size at first maturity (Brosset et al., 2017; Albo-Puigserver et al., 2018; Ramírez et al., 2021) and ultimately, in fish health status. Certain hypotheses have been confirmed about the environmental agents that are having the mentioned effects in the resource, such as fishing pressure, the increase in temperature as a result of the global warming, and their combination (Ramírez et al., 2018; Fernández-Corredor et al., 2021). Furthermore, biological factors such as food availability or parasites have been proposed as potential agents behind the drop in condition in fish. Studies have pointed at the productivity and composition of the zooplankton to be related to sardine status (El Mghazli et al., 2020; Mercado et al., 2007). However, a gap of information is found regarding the relationship between nematode parasitism and this small pelagic. Piscivorous species such as hake and cod are usually more heavily infected by ascaridoid nematodes compared to strict plankton feeders such as sardine, since abundance and spatial distribution of larvae seems largely to depend on fish host species and their respective feeding behaviour (Mattiucci et al., 2018). Thereby, the principal focus on ascaridoid parasitism has been mainly on the former, being less studied in planktivorous species as sardine, which feeds principally on zooplankton (mainly copepods, but also cladocerans, euphausids, crustacean larvae or anchovy eggs, among others), although phytoplankton is also consumed (Palomera et al., 2007; Rello et al., 2008). Nevertheless, as parasitism may affect host physiology, morphology, reproduction and behaviour (Timi and Poulin, 2020), it must be considered as a potentially determining factor in the state of health and population dynamics of the stocks, and especially the context of vulnerability in which sardines are found. Similarly, the inverse relationship between parasitism and sardine condition has been suggested, as healthier fish stocks could be considered more resistant to parasite infections (Pennino et al., 2020; Frigola-Tepe et al., 2022). Furthermore, the current state of the habitats is being altered by environmental pressures derived from global change, which could also be conditioning and altering the dynamics and life cycles of ascaridoid nematodes. In fact, warming of coastal waters potentially result in a higher number of pelagic fish species that follow warmer currents northwards, which would increase *Anisakis* spp. infection of fish, added to a general shift in host ranges and the introduction of pathogens into formerly uninfected regions (Klimpel and Palm, 2011).

In this way, it is of interest to analyse from an ecological perspective and taking into account the host (i.e., sardine) health parameters to evaluate the state of the fishing resource, since most studies that assess the parasitisation of this species by ascaridoids and, especially, by *Anisakis* spp., are carried out from the point of view of zoonoses and food safety (see, for example, Santos et al., 2006; Piras et al., 2014; Serracca et al., 2014; Bao et al., 2020). This occurs because the intake of the sibling species *Anisakis simplex* sensu stricto (s.s.) and *A. pegreffii* of the *A. simplex* sensu lato (s.l.) complex are the main causative agents of digestive or allergic symptoms to the human consumer if the fish that carried the larval nematode was not subjected to high temperatures or frozen previously to the ingestion (Villafruela-Cives and Henríquez-Santana, 2010; Roca-Geronès et al., 2021). In fact, human anisakiasis by European sardine consumption (either fresh, marinated or canned) has been reported over time and across countries (e.g., in Spain (López-Serrano et al., 2000; Molina-Fernández et al., 2015), Italy (Guardone et al., 2018), etc.). Nevertheless, there are other ascaridoid species that are not prone to cause these reactions in human consumers but that produce negative impacts on larvae and adult fish, as *Hysterothylacium aduncum* (Balbuena et al., 2000; Dallarés et al., 2016).

That is why this study intends to combine the mere description of the parasitic load of ascaridoid nematodes in sardines with ecological information on this small pelagic, since in this way we will be able to address both the immediate interest in terms of safety for the human consumption as well as exploring the fish resource status by linking this parasitism with the reproductive and energetic characteristics of sardine along different areas of its distribution. Within this context, the aims of this study have been (1) to characterise the nematode parasites in the European sardine along its distribution (stocks from the Atlantic and Mediterranean) by investigating the features of the infection and its occurrence throughout the seasons and (2) to relate these aspects of the infection with hosts' reproductive cycle and body condition, as well as with further biological traits and environmental information.

2. Materials and methods

2.1. Body condition and reproduction analyses

Specimens of *Sardina pilchardus* (N = 760) were collected seasonally (seasons defined as winter: January, February, March; spring: April, May, June; summer: July, August, September; autumn: October, November, December) from the end of 2019–2021 along the Southern Portugal-Gulf of Cádiz coast (Northeast Atlantic Ocean, Portuguese Waters - East (FAO fishing area division 27.9. a)), the coast of Málaga, bathed by the Alboran Sea (Mediterranean Sea, GFCM – GSA 1), Catalan Coast (Balearic Sea, GFCM – GSA 6), Trieste (Adriatic Sea, GFCM – GSA 17), and Thessaloniki (Aegean Sea, GFCM – GSA 22) by commercial fisheries (Fig. 1). Immediately after the purchase, samples were frozen at – 20 °C, which has been demonstrated that it has no significant effect on the studied parameters (Brosset et al., 2015a). Each sardine was measured (total length, TL ± 0.1 cm) and weighed (total body weight, W ± 0.01 g); eviscerated body weight, EW ± 0.01 g). Gonad (GW ± 0.0001 g) and liver (LW ± 0.0001 g) were also weighted. Body condition was obtained by the calculation of the relative condition index (Kn) (Le Cren, 1951) ($Kn = EW/\alpha \cdot TL^{\beta}$), where EW is eviscerated weight, TL is total length, and α and β are constants obtained by the regression line of the logarithms of length and mass from the samples per location. The gonadosomatic ($GSI = 100 \cdot [GW/EW]$) and hepatosomatic ($HSI = 100 \cdot [LW/EW]$) indexes were calculated. The sex of each specimen was determined macroscopically, and gonads were classified according to the criteria of Brown-Peterson et al. (2011) (i.e., immature, developing, spawning capable, actively spawning, regressing, and regenerating). Regarding the lipidic and energetic body condition, tissue fat content (%) was estimated by the average of both sides using a fish fatmeter (Distell Model FM 992, SARDINE-2 calibration), and a visual scale for fat mesenteric reserves with seven levels, in which level 1 represents invisible or thin and indistinct fat lines, and level 7 consists in fat line lobes and fish fundulus well-covered with fat (van Der Lingen and Hutchings, 2005).

2.2. Parasitological analysis

2.2.1. Parasite inspection method

Sardine gills were analysed by plain visual inspection. The stomach and intestine were opened to expose their content and the nematode detection was carefully performed using forceps and a lamp by naked eye. After, the whole digestive tract including the pyloric caeca, liver and gonads were placed into a transparent 1–3 mm plastic bag next to the flesh cut into butterfly fillets. Bags were then pressed under hydraulic pump and stored overnight at −20 °C for further inspection by UV-press method. The method is based on the fluorescence of frozen ascaridoid larvae, which allows the visual inspection of flattened/pressed and subsequently deep-frozen fish fillets or viscera under UV-light exposure at 366 nm in a darkened room (Cipriani et al., 2018; Levsen et al., 2018; Mattiucci et al., 2018). Afterwards, all parasites were counted, and their anatomical location was reported.

2.2.2. Ascaridoid identification by direct sequencing

Firstly, a visual identification of the nematode to genus level based on morphology was carried out following Moravec (1994) and Berland (1961) criteria. Then, total DNA from each specimen was extracted using a Quick-gDNA MiniPrep (Zymo Research Corp, CA, USA), following the manufacturer's protocol. The ITS region of the rDNA including the first internal transcribed spacer (ITS-1), the 5.8S gene, the

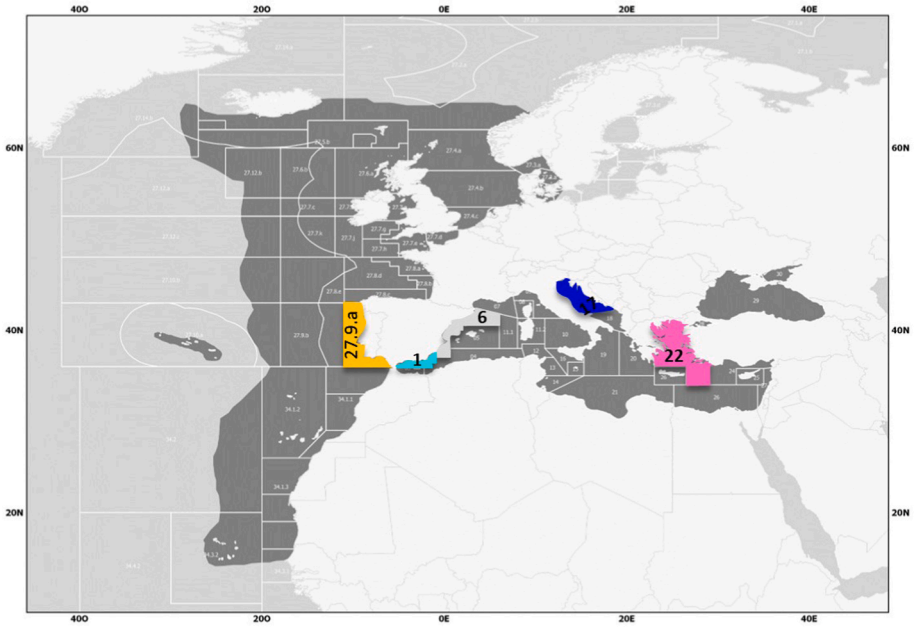

Fig. 1. Map of the European sardine (*Sardina pilchardus*) stocks sampled along its distribution (in dark grey) by subareas (FAO divisions in the Atlantic and GFCM - GSAs (into FAO Major Fishing Area 37 in the Mediterranean)). Orange: FAO Division 27.9. a Portuguese Waters - East; blue: GSA 1 Alboran; light grey: GSA 6 Northern Spain; electric blue: GSA 17 Northern Adriatic; pink: GSA 22 Aegean. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

second transcribed spacer (ITS-2), and ~70 nucleotides of the 28S gene, was amplified using the primers NC5 (forward, 5′-GTAGGTGAACCTGCGGAAGGATCATT-3′) and NC2 (reverse, 5′-TTAGTTTCTTTTCCTCCGCT-3′) as reported in Zhu et al. (2000). PCRs were carried out following the protocol reported in Palomba et al. (2021). Additionally, the mitochondrial cytochrome *c* oxidase subunit II (mtDNA *cox2*) gene locus was amplified using the primers 211F (forward, 5′-TTTTCTAGTTATATAGATTGRTTYAT-3′) and 210R (reverse, 5′-CACCAACTCTTAAAATTATC-3′). PCRs were carried out following the protocol reported in Mattiucci et al. (2014). The successful PCR products were purified, and Sanger sequenced by BioFab Research (Italy, Rome). The sequences obtained were analysed and edited by using Chromas Pro 1.34 and MEGA X v. 11 (Kumar et al., 2018). Sequence identity was checked using the Nucleotide Basic Local Alignment Search Tool (BLASTn) (Morgulis et al., 2008).

2.3. Statistical analysis

2.3.1. Analysis of the infection data

Quantitative Parasitology 3.0 software (Reiczigel et al., 2019) was applied to calculate the infection levels of *Anisakis* spp. and *Hysterothylacium* spp. larvae by sardine sampling area. General prevalence (P, %) with confidence limits (Clopper–Pearson interval, confidence level of 95%), mean intensity (mI) (Bootstrap BCa with confidence level of 95%, 2000 replications), and mean abundance (mA) (Bootstrap BCa with confidence level of 95%, 2000 replications) were obtained. The significance of statistical differences in prevalence, assessed by the Fisher's exact test, and mean intensity and abundance of larvae, analysed by bootstrap one-way ANOVA with 1000 replications, were checked by location/sardine stock. Differences were considered significant when p-value <0.05.

2.3.2. Analysis of host-parasite relationships

Using R software version 3.5.1. (R Development Core Team, 2018), Shapiro-Wilk test was applied to test the assumption of normality and Levene's test was executed to check the homogeneity of variances (Zar, 1996 in all parameters calculated. If both assumptions were met in the analysis carried out per stock comparing infected and non-infected individuals, independent samples *t*-test was performed. Conversely, when only homoscedasticity assumption was violated, data was analysed with Welch's *t*-test. For those parameters in which normal distribution was lacking but homoscedasticity was present, Kruskal-Wallis analysis of variance was applied. Chi-square test of independence was applied when testing the dependence of two categorical variables (e.g., parasite presence/absence vs. sex or mesenteric fat). The Spearman's rank non-parametric correlation test was used to explore the relationship between the number of nematodes and the sardine parameters (i.e., total length (cm), Kn, GSI (%), and HSI (%)). Statistically significant differences were considered if p-value <0.05*.

3. Results

3.1. Detection and identification of Hysterothylacium spp. and Anisakis spp. larvae

A total of 69 and 5 larval nematodes were assigned morphologically to the genus *Hysterothylacium* (Raphidascaridae family) and *Anisakis* spp. (Anisakidae family), respectively. Differences in the light reflected by the ascaridoids were appreciated under UV light: *Anisakis* spp. had an intense violet hue (Fig. 2A), while *Hysterothylacium* spp. emitted a weak yellowish light (Fig. 2B). All larvae were located in the visceral cavity of the examined sardines, mainly placed on the pyloric caeca. No parasitic individual was observed in flesh for any of the locations.

According to the sequences obtained at the ITS region of the rDNA (800 bp), 2, 3 and 38 larvae showed 100% identity with the sequences of *A. pegreffii* (accession number (a.n.) JX535520 [Mattiucci et al., 2014]), *A. simplex* (s.s.) (a.n. JX535521 [Mattiucci et al., 2014]) and *H. aduncum* (a.n. JQ934878 [Vardić Smrzlić et al., 2012]), previously deposited in GenBank. The mtDNA cox2 gene locus (580 bp), also identified the same larvae of *A. pegreffii*, *A. simplex* (s.s.) and *H. aduncum* showing >99% identity with sequences previously deposited in GenBank (*A. pegreffii*, a. n. JQ900761 [Mattiucci et al., 2013]; *A. simplex* (s.s.), a. n. DQ116426 [Valentini et al., 2006]; and *H. aduncum*, a. n. OK338702 [Karami et al., 2022]). Sequences obtained were deposited in GenBank under accession numbers: OP975692-97 (ITS) and OP985157-62 (*cox2*).

3.2. Levels of ascaridoid infection in European sardine

Significant geographical differences in general prevalence of *H. aduncum* were observed among individuals (two-sided p-value: <0.0001***). It was present in the Northern Adriatic (prevalence of 7.6%), the Atlantic (7.5%) and the Northern Spain (3.9%), and absent in

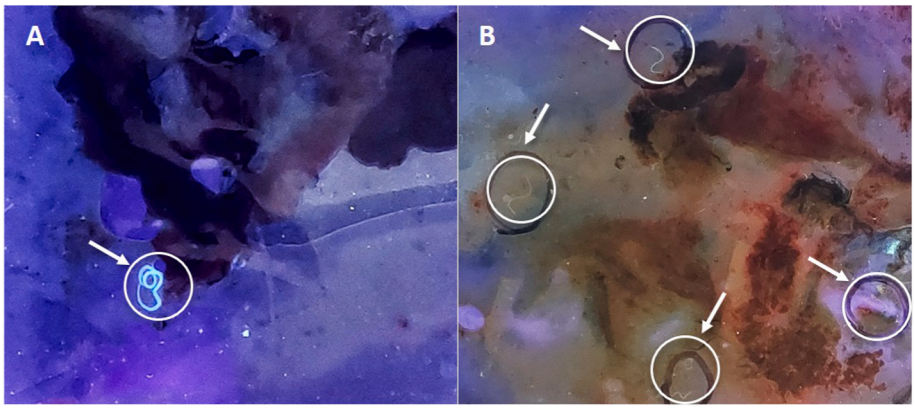

Fig. 2. Ascaridoid nematodes observed under the UV-press method. **A)** A specimen of *Anisakis simplex* (s.s.) and **B)** four individuals of *Hysterothylacium aduncum* found in sardines from the Atlantic stock (FAO Division 27.9. a Portuguese Waters – East).

the Alboran and Aegean Sea (Table 1). Prevalence and mean abundance for *H. aduncum* were significantly different by areas (two-sided p-value: <0.0001***; Bootstrap one-way ANOVA with 1000 replications resulted in p = 0.037*, respectively). Mean intensity did not present significant differences among locations (Bootstrap one-way ANOVA with 1000 replications resulted in p = 0.333) (Table 1).

Table 1
Data on number of infected European sardines, number of nematodes recovered, epizootiological parameters (prevalence (P), mean intensity ± standard deviation, mean abundance ± standard deviation) by subareas from the Atlantic (Atl) or Mediterranean (Med).

Subarea	Infection parameters	Ascaridoid		
		Hysterothylacium aduncum	***Anisakis pegreffii***	***Anisakis simplex* (s. s.)**
South Portugal (FAO Division 27.9.a) Atl $N_{analysed\ fish} = 120$	N infected fish	9	1	2
	N larvae	35	1	3
	P (%)	7.5	0.8	1.7
	mI (± SD)	3.889 ± 5.278	1.000 ± NA	1.500 ± 0.707
	mA (± SD)	0.292 ± 1.712	0.008 ± 0.091	0.025 ± 0.203
Alboran (GSA 1) Med $N_{analysed\ fish} = 119$	N infected fish	0	0	0
	N larvae	0	0	0
Northern Spain (GSA 6) Med $N_{analysed\ fish} = 259$	N infected fish	10	0	0
	N larvae	16	0	0
	P (%)	3.9	0	0
	mI (± SD)	1.600 ± 0.843	0	0
	mA (± SD)	0.062 ± 0.347	0	0
Northern Adriatic Sea (GSA 17) Med $N_{analysed\ fish} = 131$	N infected fish	10	1	0
	N larvae	18	1	0
	P (%)	7.6	0.8	0
	mI (± SD)	1.700 ± 0.823	1.000 ± NA	0
	mA (± SD)	0.130 ± 0.502	0.008 ± 0.087	0
Aegean Sea (GSA 22) Med $N_{analysed\ fish} = 131$	N infected fish	0	0	0
	N larvae	0	0	0

Anisakis pegreffii was only observed in the Atlantic and the Northern Adriatic (prevalence of 0.8% in both stocks). Significant differences among areas were found neither for prevalence (Two-sided p-value: 0.3748) nor for mean abundance (Boostrap one-way ANOVA with 1000 replications resulted in p = 0.619). For mean intensities, within-group variability could not be assessed, therefore reliable comparison of means could not be made.

Anisakis simplex (s.s.) was only found in the Atlantic (prevalence of 1.7% in the area). Significant differences among localities were found for prevalence (two-sided p-value: 0.0491*). Differences in mean abundance were not significant (Boostrap one-way ANOVA with 1000 replications resulted in p = 0.168). For mean intensities, within-group variability could not be assessed, therefore reliable comparison of means could not be made.

No ascaridoid was observed in the Alboran or the Aegean.

Significant differences among seasons were found in the parasitised locations. In the Atlantic stock, all identified *H. aduncum* were found in winter (a prevalence of 30.0% in the sardines analysed in this season, two-sided p-value: <0.0001*). All *Anisakis* spp. in this area were also observed in winter. However, due to the low prevalence, no significant differences among seasons were obtained. In Northern Spain, significant higher prevalence of *H. aduncum* (two-sided p-value: 0.0311) was recorded in spring (9.3%), followed by summer (3.1%) and autumn (1.7%). In this location, no *H. aduncum* was observed in winter. In the Northern Adriatic, higher prevalence of *H. aduncum* was recorded in autumn (15.0%), although no significant (two-sided p-value: 0.1279), followed by winter and spring (6.7% for both seasons). However, mean intensity in autumn was significantly higher (2.167 ± 0.753 in autumn, and 1.000 ± 0.000 in winter and spring) (Boostrap one-way ANOVA with 1000 replications resulted in p = 0.046*). The only specimen of *A. pegreffii* found in the Adriatic was seen in spring.

3.3. Linking sardine condition and reproduction with nematode parasitisation by fishing ground

After the analysis of sardines' condition parameters (Table 2), significant opposite patterns between reproductive (i.e., GSI) and body fat (i.e., tissue fat content and mesenteric) indices were observed in the global analysis of the annual data (Fig. 3). Moreover, Kn was negatively related to GSI in all the stocks except in the Northern Adriatic. Kn was also significantly and positively linked to body fat parameters (Fig. 3A). It was registered a general increasing trend from winter to summer regarding sardine body reserves, while the GSI started to decrease from winter on, recovering about summer and autumn months, although

Table 2
Summary of the sampled individuals of European sardine (*Sardina pilchardus*). N: number of total individuals and the identified by sex (F, female or M, male); Kn: Le Crens's index; GSI: gonadosomatic index; HSI: hepatosomatic index; N_{coll}: collected ascaridoid larvae; N_{id}: identified larvae.

Area	Season	N		*Sardina pilchardus*						Ascaridoid nematodes	
		F	M	Mean length ± SD (cm)	Kn	Tissue fat content ± SD (%)	Mesenteric fat (median) (#)	GSI ± SD (%)	HSI ± SD (%)	N_{coll}	N_{id}
South Portugal (FAO Division 27.9.a) Atl	Winter	30		18.38 ± 0.86	0.83 ± 0.06	8.11 ± 2.38	1 (1–2)	5.98 ± 3.13	0.52 ± 0.37	39	34
	Feb, Mar	16	14	(16.60–20.10)	(0.70–1.01)	(4.50–12.60)		(0.27–13.14)	(0.05–1.40)		
	Spring	30		17.96 ± 0.63	1.00 ± 0.05	20.35 ± 4.07	7 (6–7)	0.62 ± 0.24	0.87 ± 0.46	0	0
	Jun	23	7	(16.90–20.10)	(0.92–1.12)	(14.25–27.90)		(0.17–1.25)	(0.28–2.36)		
	Summer	30		17.07 ± 1.56	1.08 ± 0.10	20.81 ± 2.71	7 (6–7)	0.85 ± 0.37	0.71 ± 0.35	0	0
	Sep	18	12	(14.40–19.80)	(0.85–1.28)	(12.20–27.10)		(0.22–2.01)	(0.24–1.74)		
	Autumn	30		18.39 ± 0.56	1.13 ± 0.07	19.06 ± 1.61	7 (6–7)	1.89 ± 1.40	–	0	0
	Oct	12	18	(17.40–19.70)	(1.05–1.34)	(15.65–22.85)		(0.52–5.77)			
Alboran (GSA 1) Med	Winter	20		19.24 ± 0.91	0.91 ± 0.04	8.58 ± 1.88	1 (1–2)	9.26 ± 3.32	0.24 ± 0.10	0	0
	Feb	7	13	(15.90–20.40)	(0.84–0.99)	(4.75–11.25)		(2.50–14.17)	(0.08–0.48)		
	Spring	30		16.53 ± 3.57	0.94 ± 0.06	6.72 ± 1.90	2 (1–3)	1.86 ± 1.59	0.39 ± 0.25	0	0
	Apr, Jun	11	9	(10.90–20.20)	(0.83–1.06)	(3.90–12.40)		(0.15–6.00)	(0.07–0.97)		
	Summer	39		16.18 ± 1.12	1.10 ± 0.06	16.74 ± 2.51	7 (1–7)	0.24 ± 0.19	0.81 ± 0.28	0	0
	Aug	17	21	(10.80–17.50)	(0.90–1.19)	(6.95–20.60)		(0.060–0.86)	(0.34–1.69)		
	Autumn	30		19.53 ± 0.60	1.15 ± 0.05	20.29 ± 2.42	6 (5–7)	4.56 ± 1.57	0.51 ± 0.21	0	0
	Oct	12	18	(18.40–20.50)	(1.02–1.25)	(13.00–24.55)		(1.62–8.02)	(0.23–1.12)		
Northern Spain (GSA 6) Med	Winter	60		13.41 ± 1.35	0.92 ± 0.08	5.68 ± 2.13	1 (1–4)	2.59 ± 1.89	0.50 ± 0.30	0	0
	Feb, Mar	37	23	(10.80–16.60)	(0.82–1.31)	(2.15–11.15)		(0.24–6.81)	(0.06–1.49)		
	Spring	75		13.29 ± 1.09	1.02 ± 0.07	9.70 ± 3.48	4 (1–7)	0.32 ± 0.19	0.62 ± 0.34	13	1
	May, Jun	47	26	(9.50–16.00)	(0.61–1.20)	(3.50–18.40)		(0.06–0.97)	(0.19–1.81)		
	Summer	64		13.57 ± 0.93	1.06 ± 0.07	12.43 ± 3.18	6 (1–7)	0.29 ± 0.18	0.45 ± 0.19	2	1
	Jul, Aug	41	22	(10.90–15.10)	(0.89–1.29)	(4.70–18.20)		(0.06–0.77)	(0.07–0.81)		
	Autumn	60		13.98 ± 0.73	1.00 ± 0.08	10.83 ± 3.67	4 (1–7)	2.09 ± 1.34	0.45 ± 0.19	1	1
	Oct, Nov	19	41	(12.50–15.70)	(0.88–1.27)	(4.40–17.20)		(0.16–6.66)	(0.14–0.91)		
Northern Adriatic Sea (GSA 17) Med	Winter	30		13.25 ± 0.81	0.96 ± 0.07	6.05 ± 2.48	3 (1–6)	3.73 ± 1.89	0.92 ± 0.44	2	2
	Feb	12	18	(11.90–15.90)	(0.83–1.13)	(3.10–12.95)		(1.25–8.10)	(0.36–1.97)		
	Spring	30		12.87 ± 0.46	0.94 ± 0.07	9.52 ± 3.38	3 (1–6)	0.37 ± 0.23	0.56 ± 0.26	3	3
	May	12	18	(12.10–13.90)	(0.83–1.09)	(4.30–17.40)		(0.12–1.03)	(0.12–1.13)		
	Summer	31		13.58 ± 0.78	1.05 ± 0.07	15.78 ± 2.34	7 (6–7)	0.39 ± 0.24	0.68 ± 0.31	0	0
	Sep	19	12	(12.30–15.60)	(0.96–1.33)	(11.05–21.35)		(0.06–0.90)	(0.10–1.29)		
	Autumn	40		13.37 ± 0.91	1.06 ± 0.18	8.08 ± 4.37	2 (1–6)	3.36 ± 1.58	0.30 ± 0.14	13	12
	Nov, Dec	24	16	(11.70–15.60)	(0.85–1.56)	(3.45–16.85)		(0.63–6.54)	(0.04–0.62)		
Aegean Sea (GSA 22) Med	Winter	35		12.68 ± 0.70	0.97 ± 0.07	4.75 ± 1.09	1 (1–2)	5.79 ± 1.33	0.36 ± 0.28	0	0
	Feb	8	27	(11.60–15.00)	(0.83–1.19)	(3.30–8.25)		(3.19–8.73)	(0.06–1.18)		
	Spring	31		12.78 ± 0.56	0.99 ± 0.06	9.36 ± 2.98	5 (2–6)	0.41 ± 0.35	0.71 ± 0.26	0	0
	Jun	27	4	(11.90–14.50)	(0.85–1.16)	(4.35–16.05)		(0.10–2.18)	(0.31–1.28)		
	Summer	30		13.63 ± 0.57	1.07 ± 0.06	17.35 ± 3.29	6 (2–7)	0.33 ± 0.16	0.78 ± 0.24	0	0
	Jul	19	11	(12.30–15.00)	(0.94–1.19)	(10.85–24.35)		(0.10–0.62)	(0.30–1.34)		
	Autumn	35		12.52 ± 1.21	0.98 ± 0.06	7.94 ± 3.35	3 (1–7)	0.45 ± 0.26	0.28 ± 0.14	0	0
	Oct	23	11	(10.20–14.90)	(0.86–1.16)	(2.05–15.95)		(0.09–1.10)	(0.09–0.73)		

there were peculiarities linked to the stock (Fig. 3B).

We found the largest range of actively spawners in winter (Fig. 4A), although individuals in active spawning were detected in autumn for the Alboran (13.3%), Northern Spain (48.3%) and the Northern Adriatic (85%). Moreover, in Alboran it was detected a high percentage of active spawners in spring (71.4%), and some individuals at this stage in the Aegean (3.2%), both non-infected locations. In the Atlantic and the Northern Spain, the ratio of infection by sex was similar in both sexes ($\chi^2 = 0.6975$, df = 1, p-value = 0.4036; and $\chi^2 = 0.1652$, df = 1, p-value = 0.6844, respectively). However, in the Northern Adriatic, more males than females were infected ($\chi^2 = 7.3351$, df = 1, p-value = 0.0067*). Among the infected sardines in the Atlantic, the 100% of them were active spawners (Fig. 4B). In Northern Spain, we found that the 50% of the parasitised individuals were at regressing gonadal phase, 40% at regenerating and a 10% were spawning capable individuals, but no significant difference was proven ($\chi^2 = 2.6$, df = 2, p-value = 0.2725). In the Northern Adriatic, 70% were at actively spawning phase, 10% were in gonadal regression and 20% at regenerating stage, existing significant differences among the probability of belonging to one of these stages when parasitised ($\chi^2 = 6.2$, df = 2, p-value = 0.0451*).

Differences in length have been found among sardines from different fishing grounds (Fig. 5) (min. total length of 9.5 cm in Northern Spain and max. total length of 20.5 cm in the Alboran). Mean values of total length were the highest in sardines from the Atlantic and the Alboran, while sardines of the Aegean presented the lowest values. Intermediate sizes were detected in the Adriatic and the Northern Spain (F = 524.69, p-value <2.2e-16*). The minimum total length at which parasites were detected was 12.6 cm in individuals from the Adriatic. Bigger sardines in the Northern Spain coincided with the parasitised, while in the Atlantic and the Northern Adriatic this was not observed, although infected sardines were larger in both cases. However, the number of nematodes was positively linked to the total length in the Adriatic and the Northern Spain (see Supplementary I for statistics).

Exploring the relationship between parasitism and total length seasonally in each locality, no significant differences were observed in the Atlantic in winter between parasitised and non-parasitised, with no

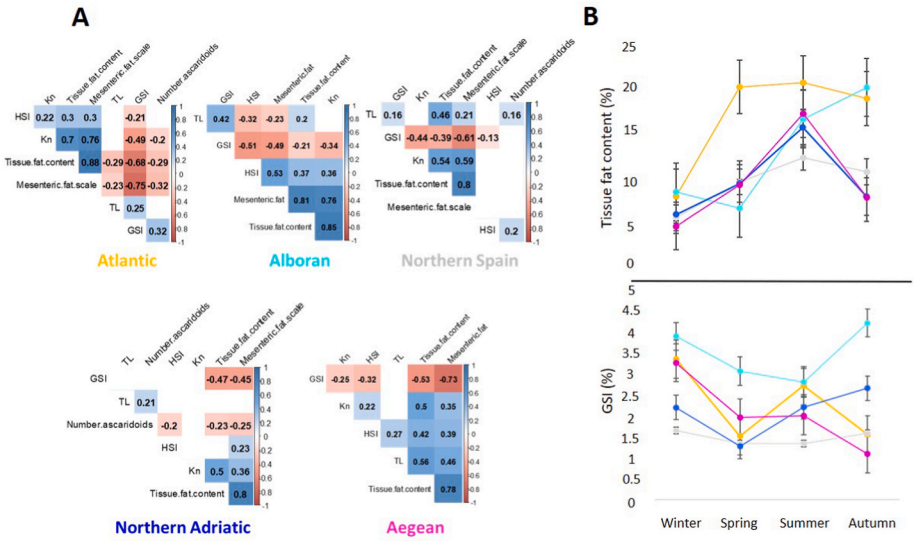

Fig. 3. A. General picture of the Spearman correlation matrixes among the European sardine's (*Sardina pilchardus*) condition indices, including the abundance of ascaridoid nematodes in the parasitised stocks (Atlantic, Northern Spain and Northern Adriatic). Total length (TL; cm), relative condition index (Kn), tissue fat content (%), mesenteric fat scale, gonadosomatic index (GSI; %), and hepatosomatic index (HSI; %) were related among each other and with the number of parasites per fish. The colour gradient from maroon to dark blue corresponds to the correlation with strength, from negative to positive, respectively. The empty squares represent a non-significant correlation according to a p value of <0.05*. **B.** Annual trends of tissue fat content (%) and GSI (%). Orange: FAO Division 27.9. a Portuguese Waters - East; blue: GSA 1 Alboran; light grey: GSA 6 Northern Spain; electric blue: GSA 17 Northern Adriatic; pink: GSA 22 Aegean. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

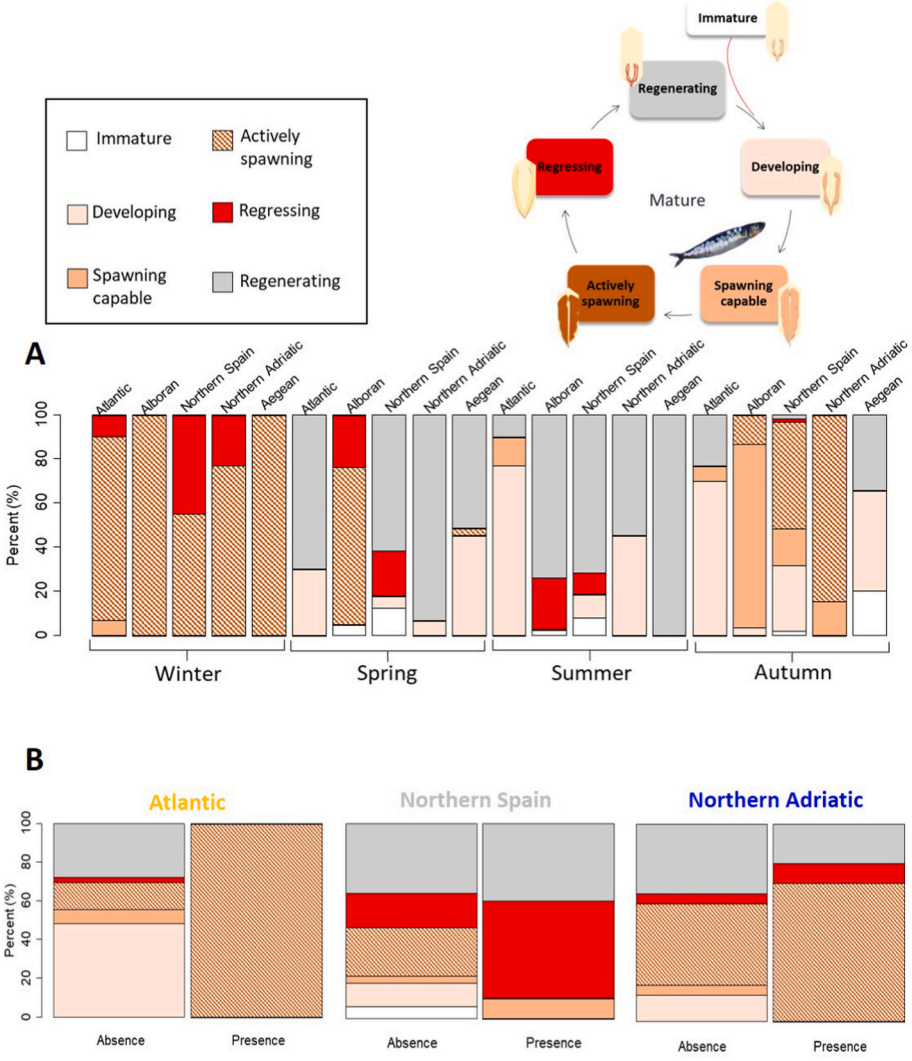

Fig. 4. A. Seasonal variations in the frequency of the reproductive developmental stages (those defined by Brown-Peterson et al., 2011) of European sardine (*Sardina pilchardus*) in the stocks analysed. **B.** Presence and absence of parasitism by reproductive developmental stage in the stocks with ascaridoids prevalence. Atlantic: FAO Division 27.9. a Portuguese Waters - East; Alboran: GSA 1; Northern Spain: GSA 6; Northern Adriatic: GSA 17; Aegean: GSA 22. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

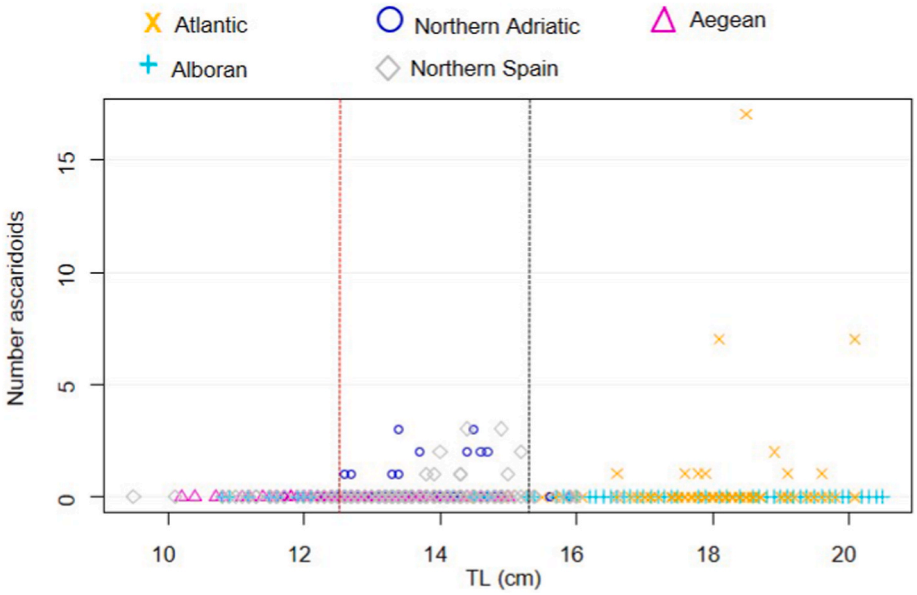

Fig. 5. Plot of European sardine (*Sardina pilchardus*) individuals by total length (cm) and its correlation with the number of ascaridoids. Orange: FAO Division 27.9. a Portuguese Waters - East; blue: GSA 1 Alboran; light grey: GSA 6 Northern Spain; electric blue: GSA 17 Northern Adriatic; pink: GSA 22 Aegean. The red dotted line shows the lowest sardine size parasitised; the black dotted line indicates the critical length from which the differences in the number of parasites cease to be significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

correlation between abundance and sardine size (see Supplementary II) (Fig. 3A). Significant differences in size between infected and uninfected sardines were recorded in the Northern Spain in spring, with bigger sardines infected (mean of 14.51 cm for infected and 13.16 cm for uninfected sardines), and also presenting a positive correlation between size and the number of parasites in this season. In the Northern Adriatic, larger sardines in autumn presented parasites (mean of 14.17 cm) when compared to uninfected individuals (mean of 13.22 cm). Moreover, in this season in the Adriatic there was a positive correlation between the number of parasites and the sardine's length.

The presence/absence and the number of nematodes seemed to be negatively related with the tissue fat content in the Atlantic, and the Northern Adriatic. However, no significant differences were shown in tissue fat content between individuals parasitised and non-parasitised in winter for the Atlantic, for Northern Spain in spring, or for Northern Adriatic in autumn, which was also confirmed by the correlation analysis at seasonal level in parasitised sardines of the Atlantic in winter, the Adriatic in autumn, and the Northern Spain in spring. Similarly, the correlation of the number of nematodes with the mesenteric fat (strongly related with the tissue fat content, see Fig. 3) showed a strong negative relationship in the Atlantic and in the Northern Adriatic, but it was not present in the Northern Spain. Nevertheless, no signification was seen when comparing mesenteric fat stage in parasitised and non-parasitised sardines in winter for the Atlantic, similar to what occurred in spring in the Northern Spain, or in autumn in the Adriatic.

Significant differences in relative condition index (Kn) were observed in the Atlantic between parasitised and non-parasitised sardines, reinforced by the Spearman correlation test, which indicated that there was a significant negative relationship between the number of nematodes and Kn for the Atlantic. Nevertheless, in winter in the Atlantic there were not significant different Kn means in infected and non-infected specimens. General values showed that no differences in Kn existed between parasitised and non-parasitised individuals in the Northern Spain. Also, in spring in the Northern Spain, Kn did not significantly vary when parasitised. In the Northern Adriatic, no differences in Kn appeared when comparing infected and non-infected sardines at global level. Nevertheless, significantly lower Kn values were seen in autumn in infected sardines of the Northern Adriatic, with values under the threshold of the good condition (0.97 (<1)), when non-parasitised reached this value (mean of 1.08). However, when exploring the correlation between the number of ascaridoids and Kn in autumn in the Northern Adriatic, it was not significant.

The number of nematodes was not related to the HSI in the Atlantic, but it seemed to be positively related in Northern Spain, and negatively in the Adriatic. Furthermore, no significant differences were found in HSI between individuals parasitised and non-parasitised in winter for the Atlantic. Also, significantly different means were quantified neither in autumn for Adriatic nor in spring for the Northern Spain.

Spearman correlation indicated a strong significant positive relationship between the number of nematodes and GSI in the Atlantic, but it was not observed for the Northern Spain or the Adriatic. Significantly higher GSI values were recorded in parasitised individuals in winter in the Atlantic (averages of 7.71% in parasitised and 5.11% in non-parasitised), accompanied by a positive correlation when studied in this concrete season. Higher GSI figures were recorded in spring in infected sardines from the Northern Spain, but without significance (mean of 0.40% in parasitised individuals and 0.32% in non-parasitised), and no correlation between GSI and the number of parasites established. A slight difference in GSI between individuals parasitised and non-parasitised was observed in autumn for Adriatic, although no significant (averages of 3.76% and 3.29%, respectively), and no correlation between GSI and ascaridoid abundance.

4. Discussion

In this study we characterised the ascaridoid parasites of the commercial small pelagic fish *Sardina pilchardus* in different fishing stocks along the Mediterranean Sea and one from the Atlantic Ocean, the southwestern Iberian Peninsula. To obtain a complete picture of the parasitisation throughout each whole individual, UV-press was used to detect the ascaridoids. This method has been considered, together with peptic digestion, the only procedures capable of providing a standardised estimate of larval ascaridoid localisation in the fish host as, in fact, the efficiency of candling and visual inspection in the detection of larvae tends to be low (Mattiucci et al., 2018). By applying this technique, our results revealed that only the third larval stage (L3) of ascaridoids was found, appearing in the digestive system and, mostly, in the pyloric caeca in all the stocks and seasons explored. We could confirm that no larva (neither *H. aduncum* nor *Anisakis* spp.) migrated to the muscle in the sardine individuals sampled. Our results are consistent with Køie (1993) and Balbuena et al. (2000), since these investigations pointed that L3 of *H. aduncum* is the larval stage mainly found in the mesenteries and digestive tract of intermediate fish hosts, like sardines, which require at least another intermediate host, a crustacean, for its

transmission to fish. The fourth larva (L4) and adult stages of *Hysterothylacium* accumulate in the stomach and intestine lumens of definitive hosts (Deardorff and Overstreet, 1981; Køie, 1993; Navone et al., 1998). In the fish stomach, larvae are activated and penetrate the stomach wall in order to seek residence in the peritoneal cavity, musculature or organs such as liver (Buchmann and Mehrdana, 2016). However, it has been also suggested that the propensity of ascaridoid larvae to migrate to the flesh may be related to differences in the nature (such as fatty acid content) of the flesh across fish species (Mattiucci et al., 2018). In fact, Smith (1984) reported that larvae migrate *post mortem* into the flesh of 'fatty' species, as sardine, but not of 'non-fatty' species. Thus, not having found ascarioids in sardines' digestive system could be explained because fish samples were immediately frozen after capture, having existed a short time for the migration to have occurred. Nevertheless, we cannot ensure that the sardine individuals would not have manifested these parasites in flesh *a posteriori*, with the problems that this would entail at the level of sale and consumption, especially of the fish from the stocks in which *Anisakis* spp. were found (South Portugal FAO Division 27.9. a and Northern Adriatic). This is because the main ascaridoid identified, *H. aduncum*, has been described as a species with neglectable/little allergenic risk for humans (Cavallero et al., 2015, 2020), although some analysis demonstrated that it shares allergens in common with the genus *Anisakis*, being associated with non-invasive anisakidosis and hypersensitive responses (Malagón et al., 2010).

For this reason, accurate identification at the species level is important to understand epidemiological patterns, but also biological and ecological, so that morphological methods are useful but often insufficient for specific identification (Roca-Geronès et al., 2018a). Thus, to assign the larvae ascaridoids to species, it was necessary to make use of molecular identification (Mattiucci and Nascetti, 2008; Aibinu et al., 2019; Pekmezci and Yardimci, 2019; Roca-Geronès et al., 2020). In fact, it was reported that visual detection at species level in *Hysterothylacium* spp. is difficult to be carried out as larval types are morphologically indistinguishable (Roca-Geronès et al., 2018b). Moreover, the inconsistency in morphological features of *Anisakis* species prompted the need to classify these nematodes by genetic and/or biochemical methods (Mattiucci and Nascetti, 2006).

Anisakis spp. and *Hysterothylacium* spp. have been previously reported in sardines from the Atlantic and Mediterranean basin (Rello et al., 2008; Gutiérrez-Galindo et al., 2010; Mladineo and Poljak, 2014; Serracca et al., 2014; Cavallero et al., 2015; Molina-Fernández et al., 2015; Bušelić et al., 2018; Frigola-Tepe et al., 2022). The comparison of these results reveals variable prevalence in sardines originating from different fishing areas, as confirmed by our study, in which, despite the differences among stocks, parasitic indices were generally low for both genus throughout the analysed area. The ecology and behaviour of the host, but also the interplay of host-parasite and host-ecosystem interactions (Brunner et al., 2017) are the responsible for the parasitic variability in fish by species, population, and region, as well as for the divergence of parasitisation in the same fish stock throughout the year. Thus, previous studies have highlighted that the prevalence of parasites in whole fish and fillets is influenced by the season, observed for *H. aduncum* and *A. simplex* (Strømnes and Andersen, 2000; Gay et al., 2018).

Plankton composition, the behaviour and distribution of the nematodes in the variable oceanographic context (i.e., the presence of upwelling or downwelling conditions) (Mattiucci et al., 2018), as well as the feeding habits of the zooplankton consumers throughout its annual cycle may condition the parasitic load in fish. Therefore, the fact that the most frequent ascaridoid in European sardine as intermediate host is *H. aduncum* and that the fish species is infrequently infected by *Anisakis* along the studied distribution suggests that, on the one hand, the invertebrate hosts of the two parasites are different and/or, on the other hand, the zooplankton from the upper layers are less parasitised by *Anisakis*, resulting in less parasitisation of pelagic clupeids (Rello et al., 2008).

In this line, no *Anisakis* spp. were characterised in sardines from the Northern Spanish Coast (GSA 6), supported by previous studies in the area (Rello et al., 2008; Gutiérrez-Galindo et al., 2010; Frigola-Tepe et al., 2022), and contradicting analyses based on morphological identification (Biton-Porsmoguer et al., 2020). Nevertheless, *H. aduncum* was observed throughout the whole year, except in winter in this location, and with much lower total prevalence and mean intensity than the 25.21% and the 2.10 obtained by Rello et al. (2008). A higher prevalence in spring was detected (9.3%), coinciding with the late winter-early spring planktonic blooms in the north-western Mediterranean (Vidussi et al., 2000; Saiz et al., 2014), to which a principal peak of zooplankton mainly constituted by copepods (72%–94% of the total zooplankton) is associated (Puelles et al., 2003). Moreover, water column seasonal stratification typically starts around April (Saiz et al., 2014), which has been shown to facilitate the transfer of *H. aduncum*, as the availability of suitable intermediate and final hosts is highest under these conditions (Klimpel and Rückert, 2005). Likewise, after the spawning period of sardine involving autumn and winter in GSA 6 (Fig. 4A), individuals started to feed intensively to recover from the reproduction investment, as well as to start to accumulate reserves for the next laying season, following the capital breeder strategy (McBride et al., 2015). In this way, it could be expected that the ability to acquire parasites through diet at the beginning of the reserve storage season is higher, as reflected in our results.

Both *A. pegreffii* and *H. aduncum* were also detected in sardine by Cavallero et al. (2015) in the Northern Adriatic Sea (2011–2012), with higher prevalence of *H. aduncum* than the reported in our analysis (42.4% and 7.6%, respectively) but lower prevalence values of *A. pegreffii* (0.2% and 0.8%, respectively). However, Bušelić et al. (2018) recorded a prevalence of 6.0% in 2013 of *A. pegreffii*, with a significant decrease in 2014 (1.0%), similar to the value obtained. Comparing to the Northern Spanish Coast, ascaridoid prevalence in the Northern Adriatic was significantly higher, probably related to production, as the Northern Adriatic Sea system is one of the major chlorophyll hot spots in the Mediterranean Sea, and it has been recognised to depend on the water and nutrient discharge from the Po River and a dozen small rivers that flow into the Adriatic Sea with a major impact on phytoplankton biomass due to the nutrients loads (Caballero-Huertas et al., 2022a).

Moreover, no individual of the genus *Anisakis* or *Hysterothylacium* were observed in the Aegean Sea throughout the year, diverging from the visual results obtained by Kuran et al. (2021), in which they observed L3 of *A. simplex* and adult individuals of *H. aduncum* in sardines. Chaligiannis et al. (2012) molecularly identified the presence of *A. pegreffii* with low prevalence (5.5%), and without signal of infection by the rest of ascaridoids analysed in this study. One potential explanation to this event may be the differences in sardine's trophic ecology among localities, as it has been documented that in the Northern Aegean Sea, sardine's diet is numerically dominated by phytoplankton (Nikolioudakis et al., 2012), while in the Atlantic and other Mediterranean locations most of their dietary energy derives from zooplankton with an occasionally substantial consumption of phytoplankton (Chen et al., 2021). In this way, the lower presence in its diet of the host prior to sardine (copepods and other crustaceans) in the area could be conditioning the intake of ascaridoids.

Ascaridoid absence in sardine occurred as well in the Alboran, as no nematode was visualised in this location. In agreement with the results of Molina-Fernández et al. (2015), neither *Anisakis* nor *Hysterothylacium* larvae were found in fish from the Alboran, which also coincided with the rate of infection in anchovy in this GSA for *A. pegreffii* (Cipriani et al., 2018). This may be supported by the fact that Alboran region is not consider a main area of downwelling (Waldman et al., 2018), unlike other locations in the Mediterranean Sea, which is known that this oceanographic event provides optimal conditions for successful recruitment of the nematode larval stage to pass to the successive intermediate/paratenic hosts (Gregori et al., 2015).

Despite its closeness with the Alboran, mainly *H. aduncum* but also

species of *Anisakis*, *A. pegreffii* and *A. simplex* (s.s.), were identified in the Atlantic waters of the Southern Iberia. Rello et al. (2008) found a prevalence, intensity, and abundance of 3.40%, 1.60 and 0.05 for *H. aduncum*, lower than the reflected by our results (7.5%, 3.889 and 0.292, respectively). This apparent growth in the infection rate could be due to temporary changes in the climate and its consequent effect on the parasite community and/or the host feeding behaviour. Nevertheless, it cannot be confirmed since the sampling season for the mentioned study is unknown and this could be conditioning the comparison between results. By contrast, the authors recognised this area as sympatric for *A. pegreffii* and *A. simplex* (s.s.) after finding these nematodes in anchovy, although the authors did not observe any of these species in sardine. This sympatric event, as well as some hybridization between the anisakids, have been previously described (Mattiucci et al., 2016; Bello et al., 2021; Roca-Geronès et al., 2021). In this stock, the highest ascaridoid intensity and abundance were characterised. As it could be observed (Fig. 5), it consisted in the parasitised stock with bigger sardines, and with the highest accumulation of fat throughout the year (Fig. 3B), sign of an abundance of resources in the area (Caballero-Huertas et al., 2022b) and a different dietary strategy (Costalago et al., 2015). Assuming that fish size increases with resources availability and age, larger and older fish are expected to accumulate a larger number of larval forms of parasites (Aco Alburqueque et al., 2020; Santoro et al., 2021). Nevertheless, total length did not seem to explain the differences in parasitism at intra-stock level in the big sardines of the Atlantic. Thus, we hypothesised that there is a critical size (>15 cm total length) in which length does not determine the amount of ascaridoids present in the sardine individual for the parasitised stocks. At intra-stock level by season, both Northern Adriatic and Northern Spain presented significant larger sizes when parasitised, with a significant positive correlation between the number of nematodes and the total length, which could reflect that in the case of stocks with medium sizes, small variations in size could be decisive in relation to their parasitic load. Thus, the low prevalence of the parasite in small, young sardines may be due to fish larvae and juvenile phenotypes feed preferentially on phytoplankton and, consequently, the youngest fish sent to market have had less opportunity than the longer ones to become infected (Rello et al., 2008).

A notable number of studies have used fish condition indices to monitor and investigate pelagic fish population health and variability (e.g., Brosset et al., 2015; Albo-Puigserver et al., 2020; Caballero-Huertas et al., 2022b). However, there are not many works that analyse nematode parasitism in the context of condition in these species, and that include the stock variability in the parasitological study of a specific species. In this context, our work has attempted to shed light on the condition of the stocks and their relationship with parasitism, as well as to study whether the individual condition of the individuals in each area can be linked to the presence of ascaridoids. In the clupeoid anchovy, lower lipid content is related with a higher intensity of certain parasites as digeneans and nematodes (Ferrer-Maza et al., 2016), also found by Frigola-Tepe et al. (2022) in sardine. Moreover, the body condition of infected cod was lower than that of those free of parasites and declined with the intensity of infection; the condition of most infected fish was up to 20% lower than that of uninfected individuals (Horbowy et al., 2016). *A. simplex* infections may be associated with the loss of condition of fish hosts, but in cases where the larvae are sequestered outside essential organs the effect may be less harmful (Buchmann and Mehrdana, 2016). Nevertheless, in our study, an apparent negative relationship could be found in terms of energy condition parameters in the parasitised stocks (Kn, and tissue and mesenteric fat in the Atlantic, and tissue and mesenteric fat in Northern Adriatic). However, when exploring at seasonal level, no differences between infected and non-infected individuals were observed in condition parameters (except for Kn for the Northern Adriatic), as well as no relationship between the abundance of parasites and fish energetic status was obtained. This reflects that the apparent relationship would be linked to seasonality and the time that the individuals of each stock go through. Fig. 4B shows clearly the link between the reproductive stage of the sardine and parasitisation, observing that sardines in active spawning and post-spawning (regressing and regenerating) were the ones that had ascaridoids of the parasitised stocks. It is noteworthy that the two non-parasitised stocks (Alboran and Aegean) corresponded to the sardines that continue to be reproductively active in spring (Fig. 3B (GSI) and 4A), so we speculate that in addition to the possible environmental explanations in the areas, perhaps this has to do with the eating habits of individuals, which could be feeding in a different way in terms of intensity and organisms by having to allocate those resources to the later egg laying. If the period of intensive feeding were closer to autumn-winter (instead of spring-summer), the main zooplanktonic bloom would not have taken place yet, in addition to the fact that water stratification and temperature would be lower, conditions that do not favour the cycle of ascaridoids or their transmission between hosts. In fact, previous studies documented the peaks of infection of *H. aduncum* and *A. simplex* in various fish species throughout the spring (Andersen, 1993; Strømnes and Andersen, 2000), moment in which actively reproducing sardines would not be feeding in the same way as the stocks that have finished spawning and are in the post-spawning recovery period (Atlantic, Northern Spain, and Adriatic).

In this work we have seen that there are multiple factors that can affect parasitisation by ascaridoids, mainly related to the ecology of the host species, its annual cycle and its way of accumulating reserves which will be destined to reproduction, which is very notable in capital breeders due to its marked seasonality. It is for all these reasons that the variability in the condition of individuals and parasitism are related due to the environmental characteristics and life history of the stocks, which determine their growth, their mode of feeding and reproducing and, consequently, the load and species of parasites that colonise them as intermediate hosts.

Declaration of competing interest

The authors declare no conflict of interest/competing interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2022.12.001.

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