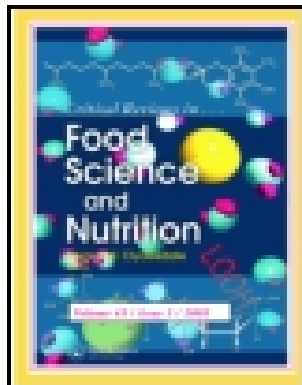


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Prevalence and Mean Intensity of Anisakidae Parasite in Seafood Caught in Mediterranean Sea Focusing on Fish Species at Risk of Being Raw-consumed. A Meta Analysis and Systematic Review

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Prevalence and mean intensity of Anisakidae parasite in seafood caught in Mediterranean Sea focusing on fish species at risk of being raw-consumed. A Meta Analysis and Systematic Review.

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Summary

Objective: to assess the prevalence and the mean intensity of anisakids in seafood caught in Mediterranean sea, focusing on fish species at risk of being raw-consumed.

Design: Systematic review and meta-analysis of studies published 1960-2012.

Study selection: main criteria for inclusion of studies were: findings of anisakids larvae, both in muscles and viscera; fish species for human consumption, caught in Mediterranean Sea;

prevalence and mean intensity data for each species; sample size equal to or more than 40 fishes.

Results: twelve studies were identified. Among them four studies considered fish species which are often consumed raw or lightly preserved or not thoroughly cooked anchovy, pilchard and Atlantic mackerel. **Data synthesis:** all pooled analyses were based on random-effect model. Anisakids prevalence in fish muscle was 0.64% ($P < 0.0001$), in viscera was 1.34% ($P < 0.0001$); overall was 0.95% ($P < 0.0001$). Mean intensity in muscle was 2.31 ($P = 0.0083$), in viscera was 1.55 ($P = 0.0174$), overall was 1.81 ($P < 0.0005$). Heterogeneity indexes (I^2) were significantly high with the exception of viscera mean intensity. **Conclusions:** anchovy, pilchard, Atlantic mackerel have a low prevalence and mean intensity of Anisakidae larvae both in viscera and in muscle. Mean Intensity is low as well.

Keywords: anisakids, Mediterranean seafood, systematic review, meta-analysis, fish consumed raw, zoonosis.

INTRODUCTION

Anisakids nematodes larvae (genera *Anisakis*, *Pseudoterranova*, *Contracaecum*, *Hysterothylacium*) are common parasites present in many marine fishes, crustaceans and squids: the problems caused by these parasites have impact both on public health (*Hysterothylacium* excepted), if they are not killed during food processing, and on commercial value of the product. Anisakiasis refers to infection of people with anisakids larvae. The Human is an accidental host in the life cycle of parasite and acquires the live larvae by eating raw or lightly preserved or undercooked seafood. Anisakiasis is a serious zoonotic disease with a number of forms depending on the localisation and the severity of lesions caused by worms. In Italy thirty one identified cases of human anisakiasis are reported over the period 1996 – 2012; in all the cases the patients referred raw or marinated fish consumption. Anisakiasis is misdiagnosed and underestimated, in fact the diagnosis is often obtained after surgery (Biondi et al., 2008; Filauro et al., 2011; Fumarola et al., 2009; Maggi et al., 2000; Mattiucci et al., 2011; Montalto et al., 2005; Moschella et al. 2004; Pampiglione et al., 2002; Pellegrini et al., 2005; Rea et al., 2008; Testini et al., 2003; Ugenti et al., 2007; Zullo et al., 2010). Although freezing and cooking assure to kill the anisakids larvae these treatments do not solve any health problem related to anisakidae presence, because of possible allergic reactions due to antigens which are freezing and cooking resistant (Pravettoni et al., 2012). The risk of allergic reactions after nonviable anisakids fish ingestion is a matter of some concern. Some authors reported that sensitized subjects did not show any symptom after administration of dead larvae (Alonso-Gómez et al., 2004; Sastre et al., 2000; Alonso et al., 1999). On the contrary, other researchers showed that 13 of 64 subjects with *Anisakis* sensitization history had adverse reaction after eating properly

cooked fish (Moneo et al., 2007). In the last study the authors believe that the intolerance recorded could be due to the high amounts of allergens. This systematic review and meta-analysis is aiming to assess prevalence (percentage of infected fishes number on total fishes number) and mean intensity (ratio of parasites number on infected fishes number) of anisakids larvae belonging to genera *Anisakis*, *Pseudoterranova*, *Contracaecum*, *Hysterothylacium*) and hosted by fishes for human consumption captured in Mediterranean Sea.

METHODS

Eligibility criteria

Criteria for eligibility of studies are described here below. Presence of anisakids larvae (genera *Anisakis*, *Pseudoterranova*, *Contracaecum*, *Hysterothylacium*: not all these genera have the same human pathogenic potential but they are morphologically very similar on visual examination). Findings of larvae, both in muscles and viscera, detected by visual and/or enzymatic digestion methods (it is strategic to know the anatomical localisation owing to the epidemiologic implication of Anisakiasis). Fish species of legal size for human consumption, caught in Mediterranean Sea and not aquacultured. Prevalence and mean intensity data for each species. Sample size equal to or more than 40 fishes: this sample size was chosen as the best compromise between a suitable estimate of parasitological indices and the sample costs; in fact, a systematic underestimation of mean intensity was demonstrated with a small sample size (<40) (Marques and Cabral, 2007), although, in metazoan parasitology, the prevalence is a variable less affected by sample size than mean intensity, and a sample size of more than 15 specimens is enough to get a basic estimate of prevalence (Jovani and Tella, 2006); moreover

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larger sample size is required for a robust estimate of prevalence. Papers published in English, French, Italian, Spanish, German and Portuguese are eligible.

Search strategy

Studies were identified by searching electronic databases and scanning reference lists of articles. This search was applied to PubMed, Embase, Web of Science, CABI, Scirus, Scopus, FSTA Food Science and Technology Abstract, Google Scholar, Medline in process, Medline Ovid, Agris FAO, Caris (FAO), SpringerLink, Annual Reviews, Conference Papers, CAB Reviews and CINAHL. Strategy has been developed using the following key words structure: “(anisakidae or anisakis or pseudoterranova or contraecum or hysterothylacium) and (prevalence or mean intensity) and Mediterranean and (fish or seafood) and larvae”. This syntax was occasionally recombined - without violating its logical sense - to meet settings of a particular database. Additionally hand searches of the reference lists of CB and of two degree theses were performed.

Study selection

The literature search was conducted independently by 2 investigators (MC and CB). Two authors (MC and CB) independently selected potentially eligible studies for inclusion. Disagreements between reviewers were resolved by consensus; if no agreement could be reached, it was planned a third author (PC) would decide. The full eligible citations was examined in more detail.

Data Extraction

For each study, prevalence and mean intensity values were extracted by 2 authors using predefined data fields, as described here below.

We developed a data extraction sheet, pilot-tested it on five randomly selected included studies, and refined it accordingly. One review author (CB) extracted from included studies and entered in the data extraction form: host fish species, fish size and fishing area; anisakids category (raw group names were collected and recorded in categories defined according to the definition set by the authors) and its anatomical location; were also recorded: sample size, number of infested fishes, number of parasites, prevalence and mean intensity. Prevalence was recorded as number of infested fishes and total number of fishes; if the number of infested fishes was missing this outcome was derived by other suitable data; mean intensity was recorded as the ratio of total number of parasites on number of parasitized fishes; if any data were missing mean intensity derivation method was analogue to that used for prevalence.

A second author checked the extracted data (FC) to ensure data quality. Disagreements were resolved by discussion between the two review authors; if no agreement could be reached, it was planned a third author would decide (PC).

The primary outcomes were the anisakids category prevalence and mean intensity.

Methodological Quality Assessment

Due to the observational nature of considered studies no methodological quality assessment method (namely about the risk of bias in individual studies or across studies or other quality items) was set.

Data analysis

Punctual estimates and their 95% confidence intervals were calculated across all selected studies on statistical units that were every level combination of the following factors: host species by parasite category by anatomical location. Calculations were performed using the

“metaprop” and “metagen” procedures of “meta” R package (Schwarzer, 2010) with the normalizing natural logarithm conversion option and, where applicable, a correction factor for 0% outcomes was introduced (i.e. a value of 0.5 was added to both numerator and denominator). Using the same R package meta-analyses were performed using the random effects model, described by DerSimonian and Laird (1986), that was selected over the fixed effects model because it incorporates within and between study variability, which is applicable to this meta-analysis that was expected to yield a high degree of variability. The chosen level of significance for statistical tests was $P < 0.05$. Heterogeneity, i.e. variability among records, was assessed by the I-squared (I^2) statistic (Higgins et al., 2003). Ninety-five per cent (95%) prediction intervals were calculated by means of “metafor” R package (Viechtbauer, 2010). Adjusted prediction intervals of mean intensity were also calculated (Zhou et al, 2007) to give more sense to the interval values, meeting the left-truncation to 1 (or 0 referring to logarithm) condition of mean intensity distribution.

RESULTS

Searches yielded 1734 references, 1683 items from electronic databases, 51 items from hand searches of the reference lists of CB. Records excluded on the basis of title and abstract 1669, references left 65.

We excluded 53 studies because they didn't meet the adopted criteria.

Twelve studies were included in quantitative synthesis of systematic review; the process of studies selection is outlined in figure 1. Four of them considered fish species which are often consumed raw or lightly preserved or not thoroughly cooked.

Characteristics of included studies

Tables n.1a and 1b present characteristics of the 12 included articles, summarised below.

Population

The included studies comprised 17 fish species, the total number of analyzed subjects were 3209 fishes, captured in different local fishing areas. Fishes size ranged from 9 to 60 cm.

Infection

Eleven categories of anisakid parasites were considered, belonging to three genera: *Anisakis*, *Contracaecum* and *Hysterothylacium* (*Pseudoterranova* genus was not found); all parasites categories were present both in muscle and in viscera.

Outcomes

Anisakidae parasites prevalence and mean intensity were the primary outcomes assessed in all studies. No additional outcomes were considered or recorded.

Prevalence and mean intensity of anisakids

Prevalence and mean intensity of anisakids are here presented only on the following fish species at risk of being raw-consumed: *Engraulis encrasicolus*, *Sardina pilchardus* and *Scomber scombrus*. The full of the data are shown in appendix A (prevalence) and appendix B (mean intensity).

All pooled analyses were based on random-effect models, the amount of heterogeneity in meta-analysis and prediction 95% intervals were also calculated.

Anisakids prevalence in muscle was 0.64% (0.23 – 1.75), ($P < 0.0001$); in viscera 1.34% (0.52 - 3.43), ($P < 0.0001$); overall 0.95 % (0.50 – 1.84), ($P < 0.0001$). Mean intensity in muscle was 2.31 (1.18 - 4.51), $P = 0.0083$; in viscera 1.55 (1.08 - 2.23), $P = 0.0174$; overall 1.81 (1.30 -

2.53), $P < 0.0005$. Heterogeneity indices (I^2) and P values, respectively for muscle, viscera and overall, in the prevalence were: 78.40% ($P < 0.0001$), 87.00% ($P < 0.0001$) and 83.60% ($P < 0.0001$); in the mean intensity were: 79.20% ($P = 0.0083$), 44.90% ($P = 0.1419$: not significant), 64.90% ($P = 0.009$). Prevalence and mean intensity muscle 95% prediction intervals were, respectively, 0.02 – 16.74% and 0.68 (1.16) – 7.87 (6.65), within brackets the adjusted values. Prevalence and mean intensity viscera prediction intervals were, respectively, 0.07 – 24.77% and 0.85 (1.09) – 2.83 (2.60), within brackets the adjusted values. Prevalence and mean intensity overall were respectively 0.06 – 15.63% and 0.84 (1.12) – 3.89 (3.49), within brackets the adjusted values.

Forest plots of prevalence and mean intensity meta-analyses are shown respectively in figure 2 and figure 3.

DISCUSSION

Summary of evidence

The rigorous sample size criteria adopted reduced the eligible papers, which should lead to robust results and conclusion. The studies enclosed in this work should provide high-quality evidence for meta-analysis. The careful examination of the papers revealed that the majority of the studies did not justify their sample size choice. Some studies were excluded due to lack of data; others were excluded because of the low quality of information, such as prevalence and mean intensity of larvae without any anatomical reference. Results of meta-analyses show an overall central estimate of anisakids prevalence in the order of 1 percent, this value being of the same order of magnitude both in muscle and in viscera. Mean intensity data yield a value that

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surrounds 2, this value is similar to that of viscera, whereas it is slightly lower than that of muscle. After a question raised from a Referee, a kind of sensitivity analysis (s.a.) was performed to assess the impact of larvae detection on results, as is known that visual method has lower recovery rate than enzymatic one: so meta-analyses were performed on the main data split in two subsets (“visual” and “enzymatic” groups), yielding output (data not shown) not giving a clue that results could be affected by the variable considered. On the contrary, the same s.a., when applied to appendix data, yielded higher outcome values in favour of visual method; this effect, being in the opposite direction than that expected, could be likely due to the very heterogeneous fish species considered in studies.

CONCLUSION

Implications for practice

The three considered species (anchovy, mackerel and pilchard) have been found lightly parasitized, but it is of primary importance for food safety that they undergo, before consumption, to a preventive treatment in order to kill all possible live larvae. The absence of genus *Pseudoterranova* in Mediterranean sea is confirmed since no record was found in the studies included (see tables 1a and 1b). In routine control of fishery products an high sample size is necessary in species with low prevalence to detect the positive lot.

Implications for research

The muscle prevalence near zero and the viscera prevalence near 1% of the Anisakidae larvae in the target species is suggesting to researchers to improve sample size because large sample size (100-1000) are required to detect very low prevalences (Gregory et al., 1991). Gutiérrez-Galindo

et al. (2010) recorded no presence of Anisakidae larvae in anchovy and pilchard, on the contrary in mackerel caught in the same area was recorded a 11% mean prevalence. Should be very interesting to understand if this evidence is an effect of low sample size or there is a biological phenomenon.

A total of 53 papers were not included in the analysis because they did not meet the eligibility criteria, this underlines the necessity to adopt a more rigorous experimental design in parasitological research, particularly most of works were excluded because the parasitological raw data were not stated even though the authors declared to record them. In our opinion, the importance of raw data publication is undervalued by researchers in this field since to access raw data would allow statistical comparison and elaboration. Moreover this work indicates further research is necessary on anisakids in those Mediterranean fish species proved to be highly parasitized in other fisheries areas, such as European sea bass, monkfish or hake.

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Table 1a. Included studies. Host fish species, size, fish area, anatomical localization of larvae, type of parasite and sample size.

Source	Host fish species	Host fish size (length, cm)	Anatomical localization of larvae	Type of larva	Fishing area	Sample size
Valero et Al.(2006)-a	<i>Merluccius merluccius</i>	40-52	viscera	<i>Anisakis spp.</i>	Mediterranean off southern Spain	63
			muscle	<i>Anisakis spp.</i>		
Gutierrez-Galindo et al.(2010)	<i>Engraulis encrasicolus</i>	12.3-16.5	viscera	No anisakids	Tarragona (NE Spain)	153
			muscle			
	<i>Sardina pilchardus</i>	11.5 - 19	viscera	No anisakids		
			muscle			
	<i>Scomber scombrus</i>	20.5-37.0	muscle	<i>Anisakis type I</i>		
			viscera	<i>Anisakis type I</i>		
			viscera	<i>Hysterothylacium aduncum</i>		
			viscera	<i>Contracaecum sp.</i>		
	<i>Trachurus trachurus</i>	16.2-30.2	muscle	<i>Anisakis type I</i>		
			viscera	<i>Anisakis type I</i>		
viscera			<i>Contracaecum sp.</i>			
Keser et Al.(2007)	<i>Pomatomus saltatrix</i>	Not stated	viscera	<i>Hysterothylacium aduncum</i>	Dardanelles at Çanakkale, Turkey	41
			viscera	<i>Hysterothylacium aduncum</i>		
Rello et al.(2009)	<i>Engraulis encrasicolus</i>	9,0-18,8	muscle	<i>Anisakis type I</i>	NW Alborán Sea	72
Figus et Al.(2005)	<i>Serranus cabrilla</i>	10,5-19,5	viscera	<i>Hysterothylacium sp.</i>	Gulf of Cagliari. south-west Mediterranean sea	71
Valero et Al.(2000)	<i>Micromesistius poutassou</i>	17-28	muscle	<i>Anisakis pegreffii</i>	Mediterranean region of southern Spain	301
			viscera	<i>Anisakis pegreffii</i>		
			viscera	<i>Anisakis physeteris</i>		
			viscera	<i>Hysterothylacium aduncum</i>		
Farjallah et Al.(2006)	<i>Phycis blennoides</i>	27-50	viscera	<i>Anisakis physeteris</i>	eastern Mediterranean coasts in Tunisia	272
			viscera	<i>Anisakis simplex s.l.</i>		
			viscera	<i>Hysterothylacium fabri L3</i>		
			viscera	<i>Hysterothylacium fabri L4</i>		
			viscera	<i>Hysterothylacium fabri L4</i>		
	<i>Phycis phycis</i>	30-60	viscera	<i>Anisakis physeteris</i>		
			viscera	<i>Anisakis simplex s.l.</i>		
			viscera	<i>Hysterothylacium aduncum L3</i>		
			viscera	<i>Hysterothylacium aduncum L4</i>		
			viscera	<i>Hysterothylacium fabri L3</i>		
Rello et Al.(2008)	<i>Sardina pilchardus</i>	12.2-21	viscera	<i>Hysterothylacium aduncum</i>	Northern Alboran sea	99
			muscle	<i>Hysterothylacium aduncum</i>		

			viscera	<i>Hysterothylacium aduncum</i>	Western Balearic Sea	115
			muscle	<i>Hysterothylacium aduncum</i>		

Table 1b. Included studies. Host fish species, size, fish area, anatomical localization of larvae, type of parasite and sample size.

Source	Host fish species	Host fish size (length, cm)	Anatomical localization of larvae	Type of larva	Fishing area	Sample size							
Ternengo et Al.(2009)	<i>Diplodus vulgaris</i>	Not stated	viscera	<i>Hysterothylacium fabri</i>	Bonifacio Strait Marine Reserve(Corsica Island, Mediterranean Sea)	72							
	<i>Mullus surmuletus</i>	Not stated	viscera			68							
	<i>Pagellus erythrinus</i>	Not stated	viscera			47							
	<i>Phycis phycis</i>	Not stated	viscera			40							
	<i>Scorpaena scrofa</i>	Not stated	viscera			57							
	<i>Symphodus tinca</i>	Not stated	viscera			58							
Valero et Al.(2006)	<i>Phycis blennoides</i>	18-55	muscle	<i>Anisakis physeteris</i>	Mediterranean coasts of eastern Andalusia (Southern Spain)	209							
			muscle	<i>Anisakis simplex</i>									
			viscera	<i>Anisakis physeteris</i>									
			viscera	<i>Anisakis simplex s.l.</i>									
			viscera	<i>Hysterothylacium aduncum</i>									
			viscera	<i>Hysterothylacium fabri</i>									
	<i>Phycis phycis</i>	24-58	viscera	<i>Anisakis physeteris</i>		58							
			viscera	<i>Anisakis simplex s.l.</i>									
Giannetto et Al.(2006)	<i>Xiphias gladius</i>	Not stated	viscera	Anisakids	Tyrrhenian and Ionian sea	66							
							Normanno et al. (2011)	Not stated	viscera	Anisakids	Adriatic sea	45	
													<i>Merluccius merluccius</i>
													<i>Micromesistius poutassou</i>
<i>Boops boops</i>	muscle	54											
<i>Trachurus trachurus</i>	viscera	62											
<i>Sardina pilchardus</i>	muscle	53											
			viscera										

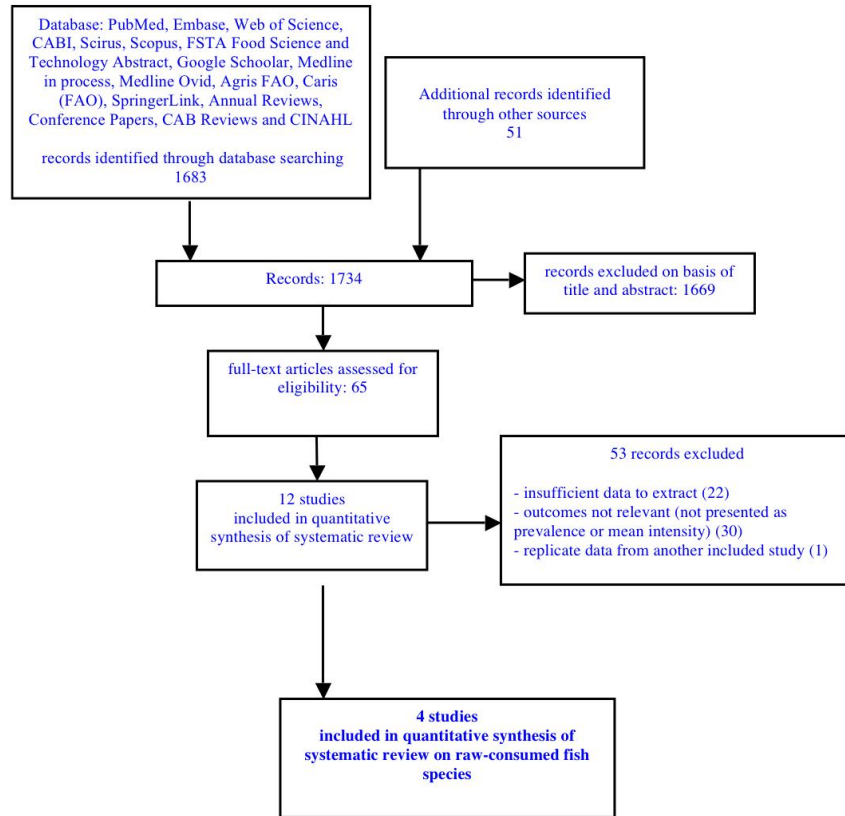


Fig 1 Selection of articles for meta-analysis

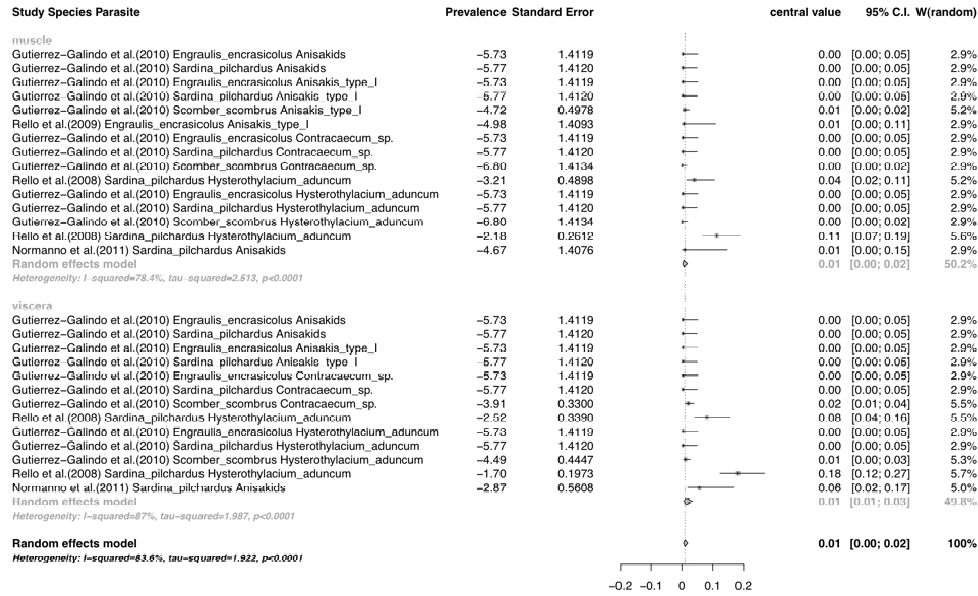


Fig 2 Forest plot of meta-analysis of anisakids prevalence on Mediterranean fish species at risk of being raw-consumed.

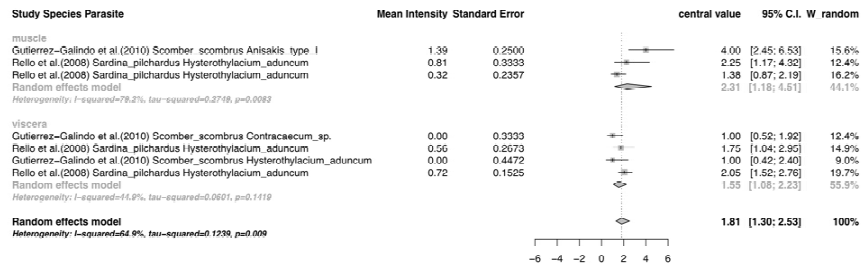
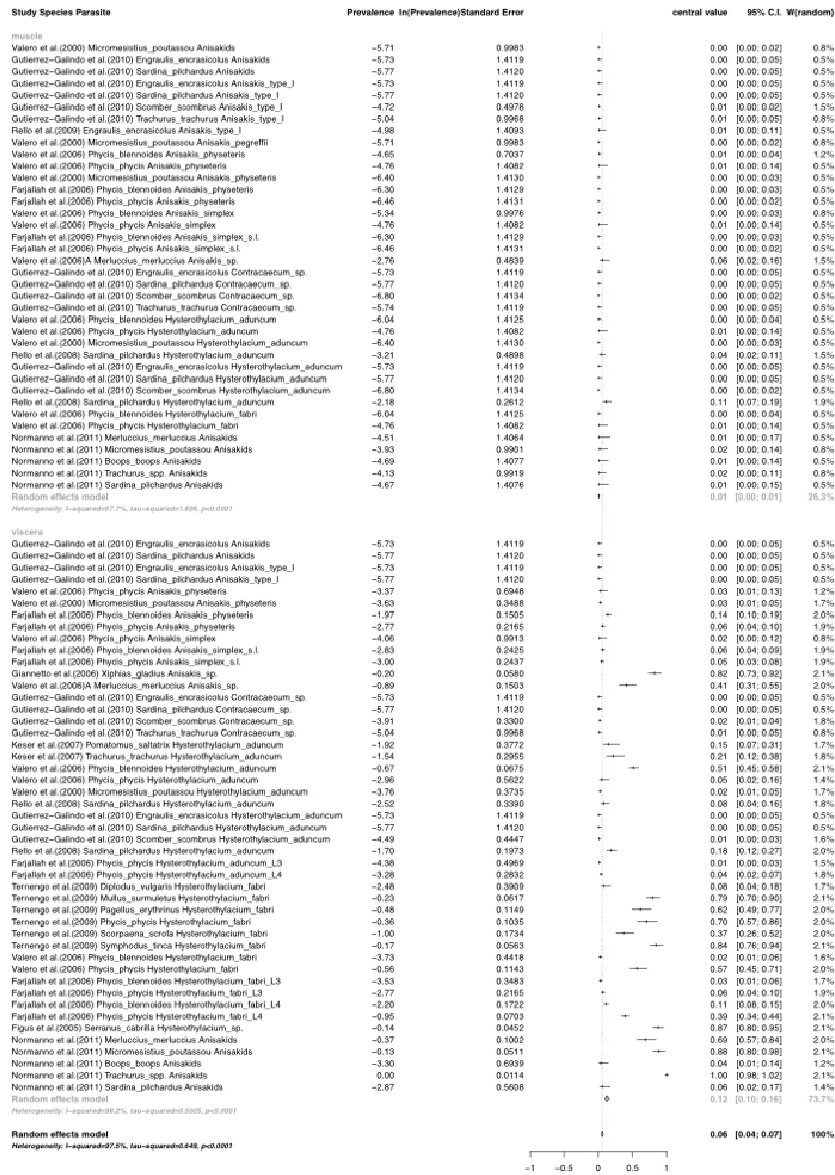
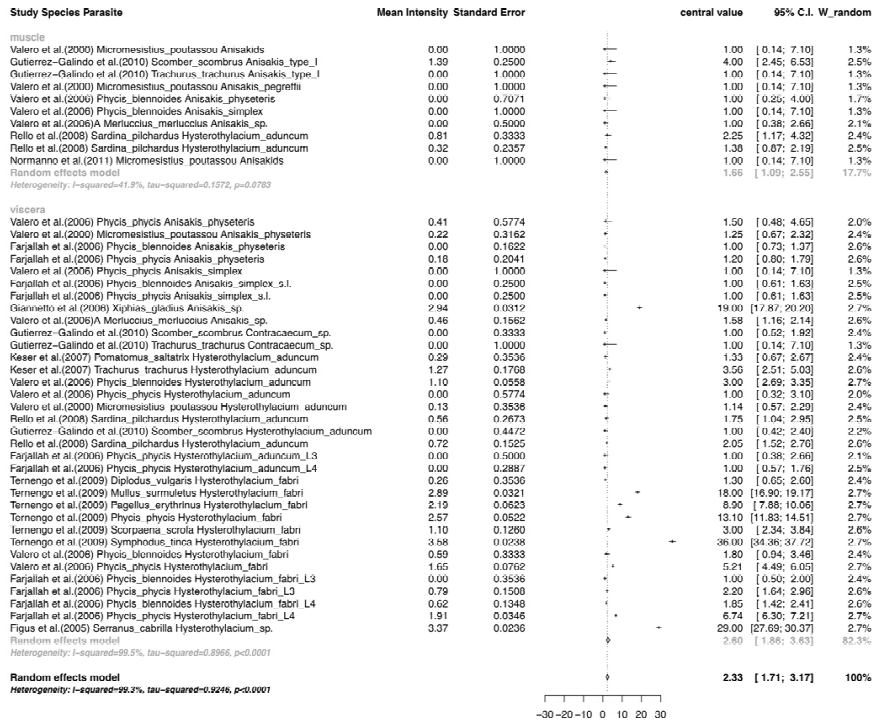


Fig 3 Forest plot of meta-analysis of anisakids mean intensity on Mediterranean fish species at risk of being raw-consumed.



Forest plot of meta-analysis of anisakids prevalence on Mediterranean fish species.



Forest plot of meta-analysis of anisakids mean intensity on Mediterranean fish species.