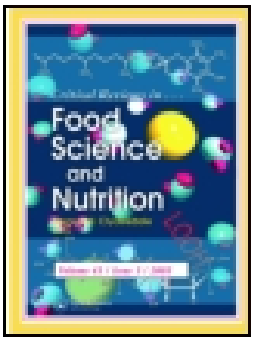

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Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

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.

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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

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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 contracaecum 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 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.

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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.

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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

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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.

Funding

This review was not funded. The authors declare no real or perceived conflict of interest

Acknowledgements

Thanks to Lorenzo Moja for extensive suggestions about systematic review and meta-analysis setup.

Thanks to Julian Higgins for precious suggestion about standard error estimator choice.

The Authors wish to thank the Referee whose comments helped to improve the manuscript.

References to studies included in this review

Farjallah, S., Slimane, B.B., Blel, H., Amor, N., Said, K. (2006). Anisakid parasites of two forkbeards (*Phycis blennoides* and *Phycis phycis*) from the eastern Mediterranean coasts in Tunisia. *Parasitol. Res.* **100** : 11-17.

Figus, V., D'Amico, V., Loddo, S.L., Siddu, N.L. & Canestri Trotti, G. (2005). Elminti parassiti di Serranus cabrilla (L.) (Osteichthyes, Serranidae) del Golfo di Cagliari, Mediterraneo sud-occidentale. *Ittiopatologia.* **2** : 207–215.

Giannetto, S., Gaglio, G. (2006). Indagini parassitologiche in specie ittiche marine selvatiche e allevate nella provincia di Messina. http://www.provincia.messina.it/ittios/pubblicazioni/Indagini_parassi.pdf

Gutiérrez-Galindo, J.F., Osanz-Mur, A.C., Mora-Ventura, M.T. (2010). Occurrence and infection dynamics of anisakid larvae in *Scomber scombrus*, *Trachurus trachurus, Sardina pilchardus,* and *Engraulis encrasicolus* from Tarragona (NE Spain). *Food Control.* **21** : 1550-1555.

Keser, R., Bray, R.A., Oguz, M.C., Celen, S., Erdogan, S., Doguturk, S., Aklanoglu, G. and Marti, B. (2007). Helminth parasites of digestive tract of some teleost fish caught in the Dardanelles at Çanakkale, Turkey. *Helminthologia.* **44** : 217-221.

Normanno, G., Dambrosio, A., Lorusso, V., Errico, L., Conversano, M. C., Convertini, M. V., Anaclerio, D., Germinario, G. L. (2011). Larve di Anisakidi in pesce pescato in Puglia: risultati preliminari. *Industrie Alimentari.* **518** : 17-22.

 ACCEPTED MANUSCRIPT

Rello, F. J., Adroher, F.J.,Benıtez, R., and Valero, A. (2009). The fishing area as a possible indicator of the infection by anisakids in anchovies (*Engraulis encrasicolus*) from southwestern Europe. *Int. J. Food Microbiol.* **129** : 277–281.

Rello, F.J., Adroher, F.J., Valero A. (2008). *Hysterothylacium aduncum*, the only anisakid parasite of sardines (*Sardina pilchardus*) from the southern and eastern coasts of Spain. *Parasitol. Res.* **104** : 117–121.

Ternengo, S., Levron, C., Mouillot, D:, Marchand, B. (2009). Site influence in parasite distribution from fishes of the Bonifacio Strait Marine Reserve (Corsica Island, Mediterranean Sea). *Parasitol. Res.* **104** : 1279-1287.

Valero, A., Martín-Sánchez, J., Reyes-Muelas, E., Adroher, F.J. (2000). Larval anisakids parasitizing the blue whiting, *Micromesistius poutassou*, from Motril Bay in the Mediterranean region of southern Spain. *J. Helminthol.* **74** : 361–364.

Valero, A., López-Cuello, M.D.M., Benítez, R., Adroher, F.J. (2006)A. *Anisakis spp.* in European hake, *Merluccius merluccius* (L.) from the Atlantic off north-west Africa and the Mediterranean off southern Spain *Acta Parasitol.* **51** : 209–212.

Valero, A., Paniagua, M.I., Hierro, I., Dıaz, V., Valderrama, M.J., Benıtez, R., Adroher, F.J. (2006). Anisakid parasites of two forkbeards (*Phycis blennoides* and *Phycis phycis*) from the Mediterranean coasts of Andalucıa (Southern Spain). *Parasitol. Int.* **55** : 1–5.

References to studies excluded from this review

Abattouy, N., Valero, A., Benajiba, M.H., Lozano, J., Martín-Sánchez, J. (2011). *Anisakis simplex s.l.* parasitization in mackerel (*Scomber japonicus*) caught in the North of Morocco--prevalence and analysis of risk factors. *International journal of food microbiology*. **150** : 136–9.

Abaunza, P., Murta, A.G., Campbell, N., Cimmaruta, R., Comesana, A.S., Dahle, G., Garcia Santamaria, M. T., Gordo, L.S., Iversen, S.A., MacKenzie, K., Magoulas, A., Mattiucci, S., Molloy, J., Nascetti, G., Pinto, A.L., Quinta, R., Ramos, P., Sanjuan, A., Santos, A.T., Strasky, C., Zimmermann, C.. (2008). Stock identity of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean Sea: Integrating the results from different stock identification approaches. *Fisheries Research*. **89** : 196–209.

Adroher, F. J., Valero, A., Ruiz-Valero, J., Iglesias, L. (1996). Larval anisakids (Nematoda: Ascaridoidea) in horse mackerel (*Trachurus trachurus*) from the fish market in Granada, Spain. *Parasitology Research*. **82** : 319–322.

Alvarez, F., Iglesias, R., Paramà, A.I., Leiro, J., Sanmartin, M. (2002). Abdominal macroparasites of commercially important flatfishes (Teleostei: Scophthalmidae, Pleuronectidae, Soleidae) in northwest Spain (ICES IXa). *Aquaculture*. **213** : 31–53.

Amor, N., Farjallah, S., Merella, P., Said, K., Ben Slimane, B. (2011). Molecular characterization of Hysterothylacium aduncum (Nematoda: Raphidascaridae) from different fish caught off the Tunisian coast based on nuclear ribosomal DNA sequences. *Parasitology research*. **109** : 1429–1437.

Anchovy Parasite Hazard Varies Depending On Origin Of Fish, Study Finds. (2011). Science daily, http://www.sciencedaily.com/releases/2009/11/091110105351.htm

ACCEPTED MANUSCRIPT

Angelucci, G., Meloni, M., Merella, P., Sardu, F., Madeddu, S., Marrosu, R., Petza, F., Salati, F. (2011). Prevalence of *Anisakis* spp. and *Hysterothylacium* spp. larvae in teleosts and cephalopods sampled from waters off Sardinia. *Journal of Food Protection*. **74** : 1769–1775.

Arcangeli, G., Mucci, N., & Cupelli, V. (2010). Exposure to *Anisakis simplex* in the fishing industry: an emerging aspect in occupational medicine? *Med. Lav.* **101** : 471–472.

Bernardi, C. (2009). Preliminary study on prevalence of larvae of Anisakidae family in European sea bass (*Dicentrarchus labrax*). *Food Control*. **20**: 433-434.

Cavallero, S., Ligas, A., Bruschi, F., D'Amelio, S. (2012). Molecular identification of *Anisakis spp*. from fishes collected in the Tyrrhenian Sea (NW Mediterranean). *Veterinary parasitology*. **187** : 563–566.

Cañás, L., Sampedro, M. P., Fariña, A.C. (2010). Influence of host biological features on macroparasites of the two European anglerfish species, *Lophius piscatorius* and *Lophius budegassa*, off North and Northwest Spain. *Journal of Parasitology*. **96** : 191–193 ST

Chai, J.Y., Darwin Murrell, K., Lymbery, A.J. (2005). Fish-borne parasitic zoonoses: Status and issues. *Int. J. Parasitol.* **35** : 1233-1254.

Chaligiannis, I., Lalle, M., Pozio, E., Sotiraki, S. (2012). Anisakidae infection in fish of the Aegean Sea. *Veterinary parasitology*. **184** : 362–6.

Chou, Y.-Y., Wang, C.-S., Chen, H.-G., Chen, H.-Y., Chen, S.-N., Shih, H.-H. (2011). Parasitism between *Anisakis simplex* (Nematoda: Anisakidae) third-stage larvae and the spotted mackerel *Scomber australasicus* with regard to the application of stock identification. *Veterinary Parasitology*. **177** : 324–331.

 ACCEPTED MANUSCRIPT

Chía, N., Romero, M. C., Polo-Vico, R., Gómez-Mateos, M., Abattouy, N., Valero, A. (2010). Estudio epidemiológico de *Anisakis* tipo I en la bacaladilla (*Micromesistius poutassou*) del noroeste de España. Epidemiological study of Anisakis type I in blue whiting (*Micromesistius poutassou*) captured in northwestern Spain. *Ars Pharm.* **51** : 829–834.

Culurgioni, J., Cuccu, D., Mereu, M., & Figus, V. (2010). Larval anisakid nematodes of *Histioteuthis reversa* (Verril, 1880) and *H. bonnellii* (Férussac, 1835) (Cephalopoda: Teuthoidea) from Sardinian Channel (western Mediterranean). *Bulletin of the European Association of Fish Pathologists.* **30** : 220–228.

Dural, M., Genc, E., Yemenicioglu, S., Sangun, M. K. (2010). Accumulation of Some Heavy Metals Seasonally in *Hysterotylacium aduncum* (Nematoda) and Its Host Red Sea Bream, *Pagellus erythrinus* (Sparidae) from Gulf of Iskenderun (North-Eastern Mediterranean). *Bull. Environ. Contam. Toxicol.* **84**: 125–131.

Dzikowski, R., Paperna, I., and Diamant, A. (2003). Use of fish parasite species richness indices in analyzing anthropogenically impacted coastal marine ecosystems. *Helgol. Mar. Res.* **57**: 220–227.

Farjallah, S., Slimane, B. B., Busi, M., Paggi, L., Amor, N., Blel, H., Said, K., and D'Amelio, S. (2008). Occurrence and molecular identification of *Anisakis* spp. from the North African coasts of Mediterranean Sea. *Parasitol. Res.* **102**: 371–379.

Fernandez de Corres, L., Del Pozo, M.D., Aizpuru, F. (2001). Prevalencia de la sensibilizacion a *Anisakis simplex* en tres areas espanolas, en relacion a las diferentes tasas de consumo de pescado. Relevancia de la alergia a *Anisakis simplex*. *Allergol. Inmunol. Clin.* **16**: 337–346.

 ACCEPTED MANUSCRIPT

Fleming, L.E., Katz, D., Bean, J.A., Hammond, R. (2001). The epidemiology of seafood poisoning. **In**: Seafood and Environmental Toxins, pp. 287–310. Hui, Kits, Stanfield, eds., New York:Marcel Dekker.

Gradassi, M. (2005). Anisakiasi ittica: indagine in pesce azzurro pescato nel Mar Adriatico centro-settentrionale. pp. 1-98. Graduate thesis, Università di Bologna Alma Mater Studiorum.

Hubert, B., Bacou, J., and Belveze, H. (1989). Epidemiology of human anisakiasis: incidence and sources in France. *An. J. Trop. Med. Hyg.* **40** : 301-303.

Jeebhay, M.F., Robins, T.G., Miller, M.E., Bateman, E., Smuts, M., Baatjies, R., and Lopata, A.L. (2008). Occupational Allergy and Asthma Among Salt Water Fish Processing Workers. *Am. J. Ind. Med.* **51** : 899–910.

Kalay, M., Dönmez, A. E., & Koyuncu, C. E. (2009). Seasonal variation of *Hysterothylacium aduncum* (Nematoda: Raphidascarididae) infestation in sparid fishes in the Northeast Mediterranean Sea. *Turk. J. Vet. Anim. Sci.* **33** : 517–523.

Karl, H., Levsen, A. (2011). Occurrence and distribution of anisakid nematodes in Grey gurnard (*Eutrigla gurnardus* L.) from the North Sea. *Food Control*. **22** : 1634–1638.

Kellermanns, E., Klimpel, S., and Palm, H.W. (2009). Parasite fauna of the Mediterranean grenadier *Coryphaenoides mediterraneus* (Giglioli, 1893) from the Mid-Atlantic Ridge (MAR). *Acta Parasitologica* **54** : 158–164.

Kleinertz, S., Klimpel, S., Palm, H. W. (2011). Parasite communities and feeding ecology of the European sprat (*Sprattus sprattus* L.) over its range of distribution. *Parasitology Research*. **110**: 1147-1157.

 ACCEPTED MANUSCRIPT

Kleter, G.A., Prandini, A., Filippi, L., Marvin, H.J.P. (2009). Identification of potentially emerging food safety issues by analysis of reports published by the European Community's Rapid Alert System for Food and Feed (RASFF) during a four-year period. *Food Chem. Toxicol*. **47** : 932-950

Llarena-Reino, M., Gonzalez, A.F., Vello, C., Outeirino, L., Pascual, S. (2012). The accuracy of visual inspection for preventing risk of *Anisakis* spp. infection in unprocessed fish. *Food Control*. **23** : 54–58.

Losito, P., Ianieri, A., Mattiucci, S. (2003). On the occurrence of *Anisakis pegreffii* (Nematoda: Anisakidae) larvae in *Engraulis encrasicolus* (L. 1758) from Adriatic sea. *Atti della Società Italiana delle Scienze Veterinarie*. **57** : 215-216.

MacKenzie, K., Campbell, N., Mattiucci, S., Ramos, P., Pinto, A.L., Abaunza, P. (2008). Parasites as biological tags for stock identification of Atlantic horse mackerel *Trachurus trachurus* L. *Fish. Res.* **89** : 136-145.

Macpherson, C.N.L. (2005). Human behaviour and the epidemiology of parasitic zoonoses. *Int. J. Parasitol.* **35** : 1319-1331.

Manfredi, M. T., Crosa, G., Galli, P., Ganduglia, S. (2000). Distribution of *Anisakis simplex* in fish caught in the Ligurian Sea. *Parasitology Research*. **86** : 551–553.

Manfredi, M.T., Marone, M., Traldi, G. (1994). Anisakidae nei prodotti ittici importati: dopo la caduta delle barriere doganali nella CE quale ruolo per il servizio sanitario nazionale? *Documenti veterinari*. **9** : 49-53.

Mattiucci, S., Abaunza, P., Ramadori, L., Nascetti, G. (2004). Genetic identification of *Anisakis* larvae in European hake from Atlantic and Mediterranean waters for stock recognition. *J. Fish Biol*. **65** : 495-510.

Mattiucci, S., Nascetti, G. (2007). Genetic diversity and infection levels of anisakid nematodes parasitic in fish and marine mammals from Boreal and Austral hemispheres. *Vet. Parasitol*. **148** : 43-57.

Meloni, M., Angelucci, G., Merella, P., Siddi, R., Deiana, C., Orrù, G., & Salati, F. (2011). Molecular Characterization of Anisakis Larvae from Fish Caught Off Sardinia. *Journal of Parasitology*. **97** : 908–914.

Mladineo, I., Simat, V., Miletić, J., Beck, R., Poljak, V. (2012). Molecular identification and population dynamic of *Anisakis pegreffii* (Nematoda: Anisakidae Dujardin, 1845) isolated from the European anchovy (*Engraulis encrasicolus* L.) in the Adriatic Sea. *International Journal of Food Microbiology*. **157** : 224–229.

Morsy, K, Bashtar, A. R., Abdel-Ghaffar, F., Mehlhorn, H., Al Quraishy, S., El-Mahdi, M., Al-Ghamdi, A., Mostafa, N. (2012). First record of anisakid juveniles (Nematoda) in the European seabass *Dicentrarchus labrax* (family: Moronidae), and their role as bio-indicators of heavy metal pollution. *Parasitology Research*. **110** : 1131-1138.

Pacini, R., Panizzi, L., Galleschi, G., Quagli, E., Galassi, R., Fatighenti, P., Morganti R. (1993). Presenza di larve di anisakidi in prodotti ittici freschi e congelati del commercio. *Industrie alimentari*. **32** : 942-944.

 ACCEPTED MANUSCRIPT

Psomadakis, P. N., Stefanni, S., Merella, P., Ferrando, S., Amato, A., & Vacchi, M. (2012). Additional records of *Beryx splendens* (Osteichthyes: Berycidae) from the Mediterranean Sea, with notes on molecular phylogeny and parasites. *Italian Journal of Zoology*. **79** : 111-119.

Pérez-del-Olmo, A. (2008). Biodiversity and structure of parasite communities in *Boops boops* (Teleostei: Sparidae) from the Western Mediterranean and off the North East Atlantic Coast of Spain. Doctorate thesis, 1-205.

Pérez-del-Olmo, A., Morand, S., Raga, J. A., Kostadinova, A. (2011). Abundance-variance and abundance-occupancy relationships in a marine host-parasite system: the importance of taxonomy and ecology of transmission. *International journal for parasitology*. **41** : 1361–1370.

Puente, P., Anadón, A.M., Rodero, M., Romarís, F., Ubeira, F.M., Cuéllar, C. (2008). *Anisakis simplex*: the

high prevalence in Madrid (Spain) and its relation with fish consumption. *Exp. Parasitol.* **118** : 271–274.

Rello, F J, Valero, A., and Adroher, F. J. (2008). Anisakid parasites of the pouting (*Trisopterus luscus*) from the Cantabrian Sea coast, Bay of Biscay, Spain. *Journal of Helminthology*. **82** : 287–291.

Renon, P., Malandra, R. (1991). Anisakidosi. Indagine sulla presenza di larva di anisakidi in teleostei marini catturati nei mari italiani. *Documenti Veterinari*. **12** : 73-79.

Renon, P., Malandra, R. (1993). Frequenza di infestazione dell’anisakidosi in teleostei marini pervenuti presso al mercato ittico di Milano nel triennio ’91-’ 93. *Archivio Veterinario Italiano.* **44**: 118-129.

ACCEPTED MANUSCRIPT

Rigos, G., Katharios, P. (2010). Pathological obstacles of newly-introduced fish species in Mediterranean mariculture: a review. *Rev. Fish Biol. Fisheries.* **20**: 47–70.

Solas, M.T., Garcia, M.L., Rodriguez-Mahillo, A., Gonzalez-Muñoz, M., De las Heras, C., Tejada, M. (2008). Anisakis antigens detected in fish muscle infested with *Anisakis simplex* L3. *J. Food Protect.* **71** : 1273–1276.

Ternengo, S., Levron, C., Mouillot, D., Marchand, B. (2009). Site influence in parasite distribution from fishes of the Bonifacio Strait Marine Reserve (Corsica Island, Mediterranean Sea). *Parasitology research*.**104** : 1279-1287.

Wharton, D.A., and Aalders, O. (2002). The response of *Anisakis* larvae to freezing. *J. Helminthol.* **76** : 363–368.

Additional references

Alonso, A., Moreno-Ancillo, A., Daschner, A., López-Serrano, M.C. (1999). Dietary assessment in five cases of allergic reactions due to gastroallergic anisakiasis. *Allergy*. **54** : 517-20.

Alonso-Gómez, A., Moreno-Ancillo, A., López-Serrano, M.C., Suarez-de-Parga, J.M., Daschner, A., Caballero, M.T., Barraco, P., Cabanas, R. (2004). *Anisakis simplex* only provokes allergic symptoms when the worm parasitises the gastrointestinal tract. Parasitol. Res. **93** : 378-84.

Biondi, G., Basili, G., Lorenzetti, L., Prosperi, V., Angrisano, C., Gentile, V., Goletti, O. (2008). Addome acuto da anisakidosi ileale. *Chirurgia Italiana*. **60** : 623-626.

DerSimonian, R., Laird, N. (1986). Meta-analysis in clinical trials. *Control. Clin. Trials*. **7** : 177-188.

Filauro, M., Rollandi, G.A., Cassola, G., Quilici, P., Angelini, G., Belli, F., Boccardo, C. (2011). Gastrointestinal bleeding due to suspected anisakiasis: challenging differential diagnosis for a rare disease. *Updates in Surgery.* **63** : 213-217.

Fumarola, L., Monno, R., Ierardi, E., Rizzo, G., Giannelli, G., Lalle, M., Pozio, E. (2009). *Anisakis pegreffi* etiological agent of gastric infections in two Italian women. *Foodborne Pathogens Disease.* **6**: 1157-1159.

Gregory, R.D. and Blackburn, T.M. (1991) Parasite prevalence and host sample size. *Parasitol. Today.* **7** : 316–318.

Higgins, J.P.T., Thompson, S.G., Deeks, J.J., Altman, D.G. (2003). Measuring inconsistency in meta-analyses. *Brit. Med. J.* **327** : 557-560.

Jovani, R., Tella, J. L. (2006). Parasite prevalence and sample size: misconceptions and solution. *Trends Parasitol.* **22** : 214-218.

Mattiucci, S., Paoletti, M., Borrini, F., Palumbo, M., Palmieri, R.M., Gomes, V., Casati, A., Nascetti, G. (2011). First molecular identification of the zoonotic parasite *Anisakis pegreffii* (Nematoda: Anisakidae) in a paraffin-embedded granuloma taken from a case of human intestinal anisakiasis in Italy. *BMC Infect. Dis.* **11**: 82.

Marques, J. F., Cabral, H. N. (2007). Effects of sample size on fish parasite prevalence, mean abundance and mean intensity estimates. *J. Appl. Ichthyol.* **23**: 158-162.

Moneo, I., Caballero, M.L., Rodriguez-Perez, R., Rodriguez-Mahillo, A.I., Gonzalez-Munoz, M. (2007). Sensitization to the fish parasite *Anisakis simplex*: clinical and laboratory aspects. *Parasitol. Res.* **101** : 1051-1055.

 ACCEPTED MANUSCRIPT

Montalto, M., Miele, L., Marcheggiano, A., Santoro, L., Curigliano, V., Vastola, M., Gasbarrini, G. (2005). *Anisakis* infestation: a case of acute abdomen mimicking Crohn's disease and eosinophilic gastroenteritis. *Digest. Liver Dis.* **37** : 62-64.

Moschella, C.M., Mattiucci, S., Mingazzini, P., De Angelis, G., Assenza, M., Lombardo, F., Monaco, S., Paggi, L., Modini, C. (2004). Intestinal anisakiasis in Italy: case report. *J. Helminthol.* **78** : 271-273.

Pampiglione, S., Rivasi, F., Criscuolo, M., De Benedittis, A., Gentile, A., Russo, S., Testini, M., Villani, M. (2002). Human anisakiasis in Italy: a report of eleven new cases. *Pathol. Res. Pract.* **198** : 429-434.

Pellegrini, M., Occhini, R., Tordini, G., Vindigni, C., Russo, S., Marzocca, G. (2005). Acute abdomen due to small bowel anisakiasis. *Digest. Liver Dis.* **37** : 65-67.

Pravettoni, V., Primavesi, L., Piantanida, M. (2012). *Anisakis simplex:* current knowledge. *Eur. Ann. Allergy Clin. Immunol.* **44** :150-156.

Rea, R., Di Matteo, F., Scarpa, F., Pandolfi, M., Martino, M., Dicuonzo, G., Gabbrielli, A. (2008). Identification and removal of *Anisakis* in acute gastric anisakiasis. *Digest. Liver Dis.* **40S** : S73.

Schwarzer G. (2010). Meta-Analysis with R. R package version 1.6-0. http://CRAN.Rproject.org/package=meta.

Sastre, J., Lluch-Bernal, M., Quirce, S., Arrienta, I., Lahoz, C., Del Amo, A., Fernandez-Caldas, E., Maranon, F. (2000). A double-blind placebo controlled oral challenge study with lyophilized larvae and antigen of the fish parasite *Anisakis simplex*. *Allergy.* **55** : 560-564.

Testini, M., Gentile, A., Lissidini, G., Di Venere, B., Pampiglione, S. (2003). Splenic anisakiasis resulting from a gastric perforation: an unusual occurrence. *Int. Surg.* **88** : 126-128.

ACCEPTED MANUSCRIPT

Ugenti, I., Lattarulo, S., Ferrarese, F., De Ceglie, A., Manta, R., Brandonisio, O. (2007). Acute gastric anisakiasis: an Italian experience. *Minerva Chirurgica*. **62:** 51-60.

Viechtbauer W. (2010). Conducting meta-analyses in R with the metafor package. *J. Stat. Softw.* **36** : 1-48.

Zhou, M., Yang, D., Wang, Y., Nadarajah S.(2010). Programs in R for computing, truncated normal distributions. *Comput. Appl. Eng. Educ*. **18**: 589-592.

Zullo, A., Hassan, C., Scaccianoce, G., Lorenzetti, R., Campo, S.M.A., Morini, S. (2010). Gastric Anisakiasis: do not forget the clinical history! *J. Gastrointest. Liver Dis.* **19** : 359.

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	*Merluccius merluccius*	40-52	viscera	*Anisakis spp,*	Mediterraneanoff southern Spain	63
			muscle	*Anisakis spp,*		
Gutierrez-Galindo et al.(2010)	*Engraulis encrasicolus*	12.3-16.5	viscera	No anisakids	Tarragona (NE Spain)	153
			muscle			
	Sardina pilchardus	11.5 - 19	viscera	No anisakids		160
			muscle			
	Scomber scombrus	20.5-37.0	muscle	*Anisakis type I*		447
			viscera	*Anisakis type I*		
			viscera	*Hysterothylacium aduncum*		
			viscera	*Contracaecum sp,*		
	Trachurus trachurus	16.2-30.2	muscle	*Anisakis type I*		155
			viscera	*Anisakis type I*		
			viscera	*Contracaecum sp,*		
Keser et Al.(2007)	*Pomatomus saltatrix*	Not stated	viscera	*Hysterothylacium aduncum*	Dardanelles at Çanakkale, Turkey	41
			viscera	*Hysterothylacium aduncum*		
Rello et al.(2009)	*Engraulis encrasicolus*	9,0-18,8	muscle	*Anisakis type I*	NW AlboránSea	72
Figus et Al.(2005)	*Serranus cabrilla*	10,5-19,5	viscera	*Hysterothylacium sp,*	Gulf of Cagliari. south-west Mediterranean sea	71
Valero et Al.(2000)	*Micromesistius poutassou*	17-28	muscle	*Anisakis pegreffii*	Mediterranean region ofsouthern Spain	301
			viscera	*Anisakis pegreffii*		
			viscera	*Anisakis physeteris*		
			viscera	*Hysterothylacium aduncum*		
Farjallah et Al.(2006)	*Phycis blennoides*	27-50	viscera	*Anisakis physeteris*	eastern Mediterranean coasts in Tunisia	272
			viscera	*Anisakis simplex s,l,*		
			viscera	*Hysterothylacium fabri L3*		
			viscera	*Hysterothylacium fabri L4*		
			viscera	*Hysterothylacium fabri L4*		
	Phycis phycis	30-60	viscera	*Anisakis physeteris*		320
			viscera	*Anisakis simplex s,l,*		
			viscera	*Hysterothylacium aduncum L3*		
			viscera	*Hysterothylacium aduncum L4*		
			viscera	*Hysterothylacium fabri L3*		
			viscera	*Hysterothylacium fabri L4*		
Rello et Al.(2008)	*Sardina pilchardus*	12.2-21	viscera	*Hysterothylacium aduncum*	Northern Alboran sea	99
			muscle	*Hysterothylacium aduncum*		

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			viscera	*Hysterothylacium aduncum*	Western Balearic Sea	115
			muscle	*Hysterothylacium aduncum*		

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)	*Diplodus vulgaris*	Not stated	viscera	*Hysterothylacium fabri*	Bonifacio Strait Marine Reserve(Corsica Island, Mediterranean Sea)	72
	Mullus surmuletus	Not stated	viscera			68
	Pagellus erythrinus	Not stated	viscera			47
	Phycis phycis	Not stated	viscera			40
	Scorpaena scrofa	Not stated	viscera			57
	Symphodus tinca	Not stated	viscera			58
Valero et Al.(2006)	*Phycis blennoides*	18-55	muscle	*Anisakis physeteris*	Mediterranean coasts of eastern Andalucia (Southern Spain)	209
			muscle	*Anisakis simplex*		
			viscera	*Anisakis physeteris*		
			viscera	*Anisakis simplex s.l.*		
			viscera	*Hysterothylacium aduncum*		
			viscera	*Hysterothylacium fabri*		
	Phycis phycis	24-58	viscera	*Anisakis physeteris*		58
			viscera	*Anisakis simplex s.l.*		
			viscera	*Hysterothylacium aduncum*		
			viscera	*Hysterothylacium fabri*		
Giannetto et Al.(2006)	*Xiphias gladius*	Not stated	viscera	Anisakids	Tyrrhenian and Ionian sea	66
Normanno et al. (2011)	*Merluccius merluccius*	Not stated	viscera	Anisakids	Adriatic sea	45
	Micromesistius poutassou	Not stated	viscera			51
			muscle			
	Boops boops	Not stated	viscera			54
	Trachurus trachurus	Not stated	viscera			62
			muscle			
	Sardina pilchardus	Not stated	viscera			53

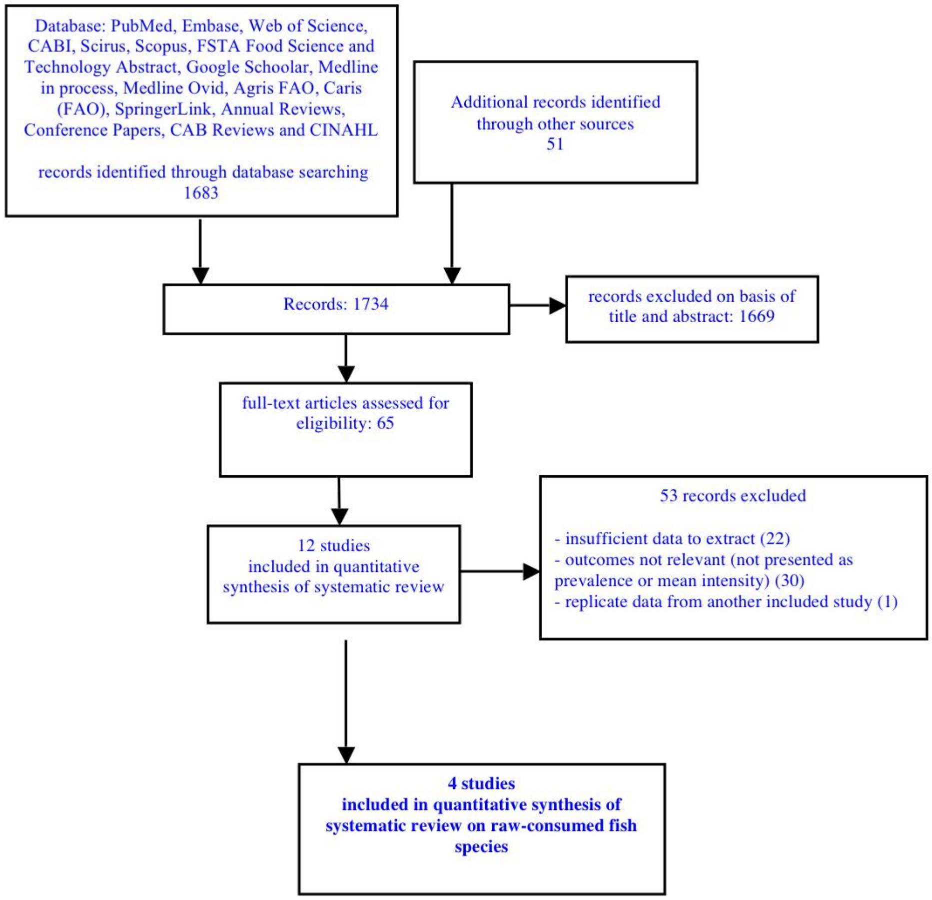

Fig 1 Selection of articles for meta-analysis

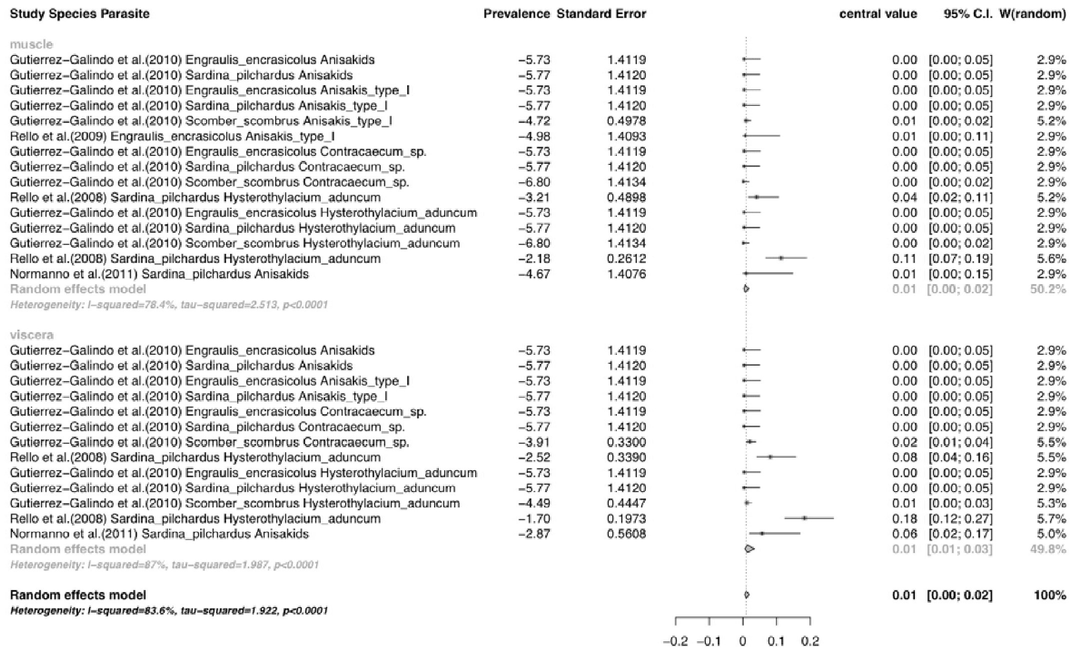

Fig 2 Forest plot of meta-analysis of anisakids prevalence on Mediterranean fish species at risk of being raw-consumed.

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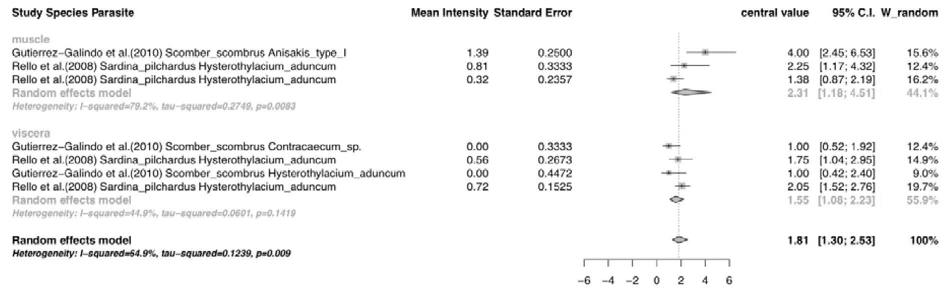
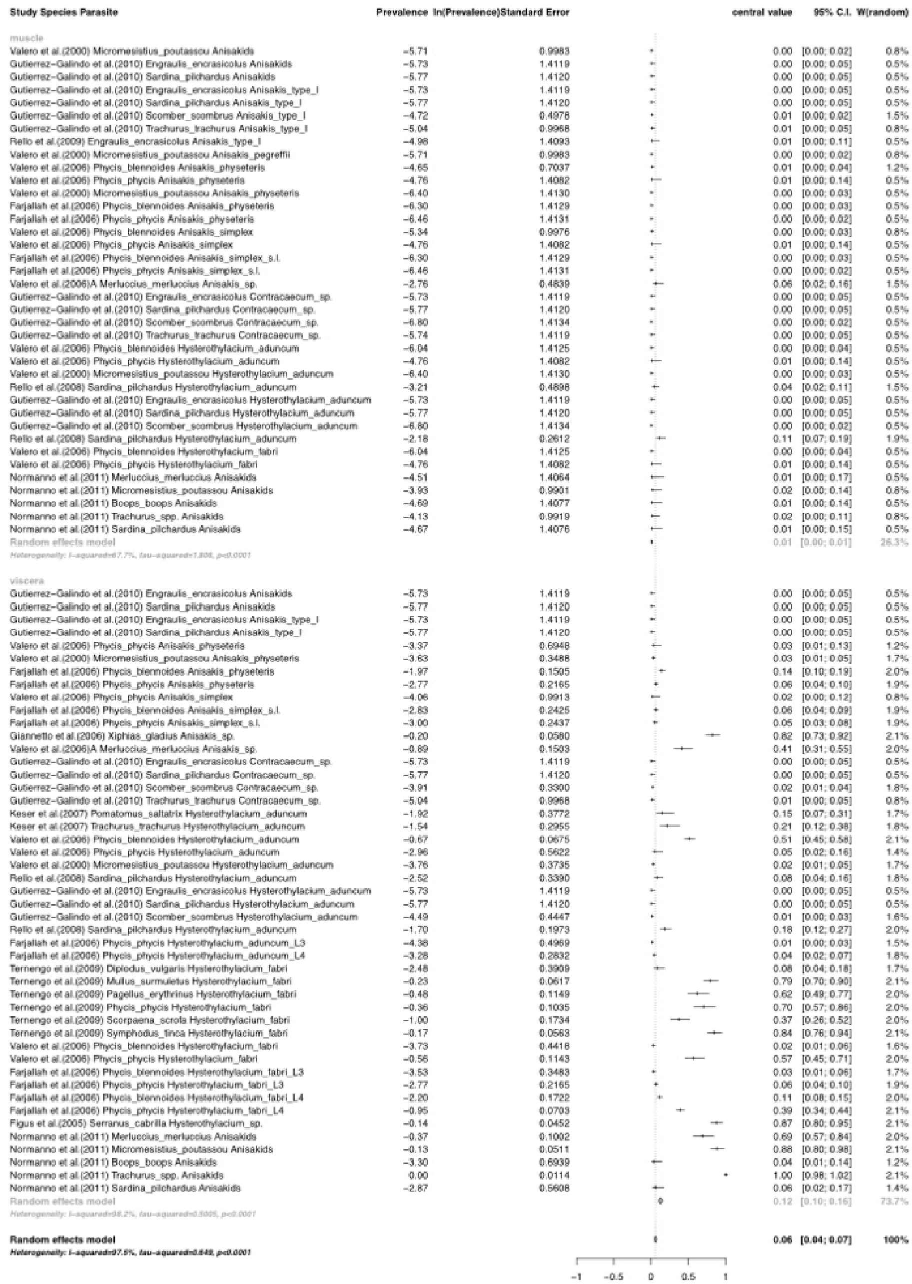
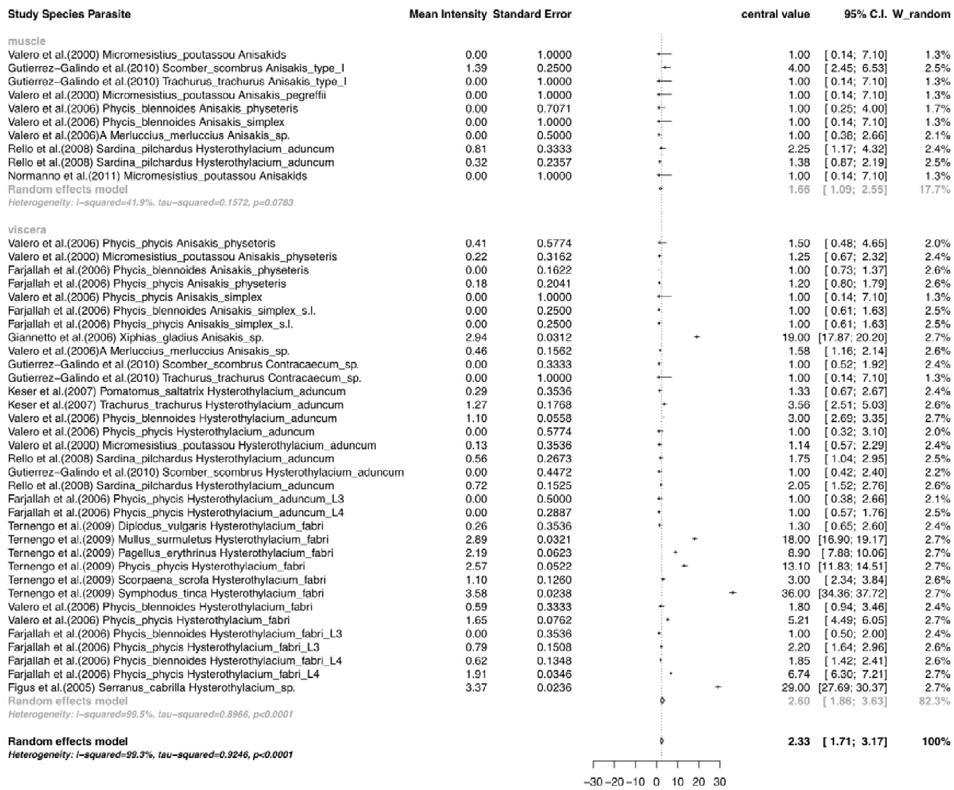


Fig 3 Forest plot of meta-analysis of anisakids mean intensity on Mediterranean fish species at risk of being raw-consumed.



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.