# Bringing up the life cycle of the *Anisakis simplex* complex (Nematoda: Anisakidae) in temperate NE Atlantic waters

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## Abstract

Third-stage larvae ($L_3$) of the parasitic nematodes *Anisakis simplex* and *Anisakis pegreffii* were isolated from euphausiids, mysids, and salpids collected in Galician waters (NE Atlantic), with an overall prevalence of infection of 0.0019%. Parasite identification was undertaken by means of morphological diagnostic characters and molecular markers using the internal transcribed spacer (ITS) region. The present work extends the intermediate host range of the *A. simplex* complex in mesozooplankton populations from temperate waters off the NE Atlantic, filling up the zooplankton gap in its life cycle in the studied area. Moreover, the present record enlarges the ramifications for the transmission of anisakids interconnecting benthic and pelagic realms. The results also suggest that the recruitment of *Anisakis simplex* complex may be affected by the oceanography, being different under upwelled or downwelled conditions. The infected mysids and salpids suggested that *Anisakis simplex* complex are not specific at mesozooplankton level, using different hosts to cross habitats and enlarge the pathways in order to find their definitive mammal hosts.

**Keywords**: *Anisakis pegreffii*, *Anisakis simplex,* third-stage larvae ($L_3$), zooplankton, NE Atlantic.

## Introduction

Nematodes of the genus *Anisakis* Dujardin, 1845 are by far one of the most important parasites in the marine ecosystem. The above “privilege” is due to the fact that it is the most cosmopolitan and prevalent marine macroparasite worldwide. Furthermore, in the last 20 years the risk of a fish production system infected with *Anisakis* have been traditionally associated with seafood security and seafood safety concerns (EFSA 2010). The ingestion of live anisakid larvae with raw or undercooked seafood can cause anisakiasis (Nagasawa 1990; Ishikura et al. 1992; Smith 1999; Nieuwenhuizen et al. 2006; Plessis et al. 2004). However, IgE-mediated reactions, with several clinical manifestations after eating well cooked but infected fish, have also been reported (Audicana et al. 1997; Caballero and Moneo 2004). Numerous reports have showed from anaphylaxis, acute urticaria or angiodema in the course of gastro-allergic anisakiasis to allergic airbone asthma and dermatitis in the domestic and occupational settling after work place exposure in the fish industry (EFSA 2010; Pontone et al. 2010; Kim et al. 2012; Shiryaeva 2012).

From an ecological perspective, the evolutionary success of a multihost complex life cycle within Anisakidae species has a marked phylogenetic basis (Mattiucci and Nascetti 2008). However, it has also important constraints related to different factors that have favoured these indirect life cycles linked to the trade-offs between transmission strategies (Poulin 1998; Choisy et al. 2003). Actually, one of the most relevant findings is the fact that anisakids infect species that are key trophic links or bridges between trophic levels in the marine ecosystems (Marcogliese 1995; Abollo et al. 1998, 2001). Therefore, the adjustment of the parasite life cycle to the trophic web is remarkable, even showing differential fitness among the host species of similar ecological value (Abollo et al. 2001). All these strategies ensure a high parasite flow of larval stages by several trophic routes from the mesozooplankton to the top predators of the marine environment. In this ecological framework, some components of the mesozooplankton, naturally infected with larval ($L_3$) of Anisakidae, have been reported worldwide (Table 1). Moreover, infected mysids have also been described by Køie (1993) who reported *Hysterothylacium aduncum* Rudolphi, 1802 from Isefjord and Marcogliese (1992, 1993) that found ascarioid nematodes in the opossum shrimp *Neomysis americana* Smith, 1873 from Sable Island pond (Eastern of Canada). In addition, Marcogliese and Burt (1993) found the spirurid *Ascarophis* sp. van Beneden, 1871 as well as *Paracuaria adunca* Creplin, 1846 in *Mysis stenolepis* Latreille, 1802 in Passamaquoddy Bay, New Brunswick. Finally, Jackson et al. (1997) reported infected mysids by *Pseudoterranova decipiens* Krabbe, 1878, *H. aduncum* and *P. adunca* in nine areas in the Northwest Atlantic. As far as we known, there is only a single report of *Anisakis simplex* in *Mesopodopsis slabberi* van Beneden, 1861 from West Scotland (Makings 1981).

The aim of this paper was to undertake the diagnosis of the third-larval stage of anisakid nematodes found in mesozooplankton communities collected off NW Iberian Peninsula waters. Furthermore, demographic and ecological findings were discussed in relation to the coastal upwelling and the structure of mesozooplankton communities in the studied area.

## Materials and Methods

*Collection and processing of parasite larvae.*

Zooplankton samples were caught in the Ría de Vigo in Galician waters (NW Spain) on board of the RV *"Mytilus"* (Figure 1). In 2008, ten surveys were undertaken, in summer (2nd, 4th, 9th and 11th of July) and autumn (26th of September; 1st, 3rd, 9th, 10th and 14th of October). Two samples were collected on each transect by double oblique towing, using a 750 mm diameter bongo net equipped with 375 µm mesh. At a ship's speed of 2 knots, the bongo net was first lowered and stabilized near the bottom for a period of 15 min, then hauled to the surface at 0.5 $ms^{-1}$. Bongo net was also equipped with a current meter to determine the filtered volume. Zooplankton samples were fixed on board with 70% ethanol and stored at -20 ºC to avoid DNA degradation (Passmore et al. 2006). The abundance of the different zooplankton taxa was calculated as described in Roura et al. (2013). In this work we studied the two samples collected at transect 5 (T5, Figure 1) on 2nd, 4th, 9th of July and 26th of September, due to the extraordinary abundance of adults of euphausiids found on it (Roura et al. 2013). Mesozooplankton was identified to the lowest taxonomic level. Species diversity was calculated using the Shannon-Wiener and Evenness Index (Omori and Ikeda 1984; Guisande et al. 2006). The number of euphausiids was estimated by the method of calculating precise replica (Andrew and Mapstone 1987).

All samples (n=8) were examined for nematodes using a stereomicroscope (20x). When nematodes were found, they were extracted from the host by dissection.

Some larvae were used for a morphological identification using features described by Hartwich (1974), Yoshinaga et al. (1987) and Berland (1989). These larvae were clarified with lactophenol, and therefore it was not possible to use them for molecular procedures. Infection parameters were estimated following Bush et al. (1997) and Rósza et al. (2000). Sterne's exact 95% confidence interval (CI) was calculated for prevalence (Reiczigel 2003).

*Molecular methods.*

Genomic DNA was isolated using Qiagen DNeasy$^{TM}$ Tissue Kit according to manufacturer's instructions. DNA quality and quantity was checked in a spectrophotometer Nanodrop$^{®}$ ND-1000 (Nanodrop technologies, Inc) and in 1% agarose gel. The entire internal transcribed spacer (ITS) (ITS1, 5.8S rDNA gene and ITS2) was amplified using the primers NC5 (forward: 5'-GTAGGTGAACCTGCGGAAGGATCATT-3') and NC2 (reverse: 5'-TTAGTTTCTTCCTCC GCT-3') (Zhu et al. 2000).

PCR reactions were performed in a total volume of 25 µl containing 1 µl of genomic DNA (100 ng), PCR buffer at 1x concentration, 1.5 mM $MgCl_2$, 0.2 mM nucleotides (Roche Applied Science), 0.3 µM primers and 0.625 U Taq DNA polymerase (Roche Applied Science). The cycling protocol was 2 min at 94 ºC, 35 cycles with 30 s at 94 ºC, 30 s at 55 ºC and 1 min 15 s at 72 ºC, followed by 7 min at 72 ºC. PCR products were separated on a 2% agarose gel (in 1x Tris-acetic EDTA buffer), stained with ethidium bromide and scanned in a GelDoc XR documentation system (Bio-Rad Laboratories). PCR products were cleaned for sequencing using the ExoSAP-IT reagent (USB Corporation) according to the manufacturer's instructions. Sequencing was performed by Secugen company (Madrid). Chromatograms were analysed using

ChromasPro version 1.5 Technelysium Pty Ltd. All generated sequences were searched for similarity using BLAST (Basic Local Alignment Search Tool) through web servers of the National Center for Biotechnology Information (USA).

## Results

The composition, number and total abundance of mesozooplankton collected as well as the Shannon-Wiener index for the entire assemblage are shown in Table 2. The samples studied in this work belonged to the frontal and oceanic communities as defined in Roura et al. (2013). Accordingly, samples collected on 2nd of July belonged to the summer frontal (SF), the samples took on 4th of July belonged to the summer oceanic community (SO) and the samples collected on surface on 9th of July belonged to the SF whereas, the sample of the column belonged to the SO community. Finally, samples collected on 26th of September belonged to the autumn oceanic (AO) community (Table 2). Among these three communities, salpids were the most abundant taxa in SO (213.75 individuals/m$^3$) followed by AO (61.48 individuals/m$^3$). By contrast, in SF euphausiids were the most abundant animals (686.25 individuals/m$^3$). Copepods were, without a doubt, the most abundant taxa in the holoplankton with 443.35, 146.88, and 33.63 copepods/m$^3$ in FS, SO and AO, respectively. Mysidacea dominated in AO and decreased from SF to SO. The abundance of krill population decreased from SF to AO and SO. However, furcilia behaved differently and did not follow the same pattern, decreasing from SF to SO and AO (Roura et al. 2013).

A total of 18 nematode $L_3$ larvae of the *Anisakis simplex* complex (Table 3) were found. According to the morphological features (Hartwich 1974; Yoshinaga et al. 1987; and Berland 1989) a total of 7 $L_3$ were identified as *A. simplex s.l.*

Molecularly, the amplification of the ITS region yielded a single 1,000 bp amplicon. BLAST search showed sequence identity values of 100% with *A. simplex s.s.* (6 larvae) and with *A. pegreffi* (5 larvae). Nucleotide sequence data reported in this paper are available on GenBank under the Accession numbers.

The average total length (TL) was 19.03 ± 3.20 mm [mean ± SD], n = 7. The smallest individual measured was 13.86 mm, while the largest one was 24.00 mm (TL). Fourteen *Anisakis* were found in SF community (Table 3). Five *Anisakis simplex s.l.* were found, one of them into *Nyctiphanes couchii*, two into *Salpa fusiformis* Cuvier, 1804 (Figure 2 A-C), and the remaining two were found free in the water column. Additionally, four *Anisakis simplex s.s.* were encountered. One of them was found laid apparently free in the haemocoel of an adult of *Nyctiphanes couchii* (Figure 2 D-F), while the others were encountered free in the water column. Finally, five *Anisakis pegreffi* were found. One of them was located in the haemocoel of an adult of *N. couchii* (Figure 2 G-I) and the rest of them were found free in the water column.

In SO community, three $L_3$ were found. Two *Anisakis simplex s.l.* were encountered, one of them floating in the water column and the other infecting a mysid (Figure 2 J-L). The last larvae belonged to *Anisakis simplex s.s.* was also found in the water column. Contrastingly, only one *Anisakis simplex s.s.* was found free in the AO community. The number of $L_3$, prevalence and abundance of *Anisakis* complex are summarized in Table 3.

## Discussion

This study represents the first investigation by survey on the larvae of *Anisakis simplex* complex in zooplankton samples collected in temperate NE Atlantic waters.

The identification of *A. simplex s.s*. and *A. pegreffi* parasitizing zooplankton samples show that both species are able to share the same intermediate euphausiid host reinforcing their sympatric distribution in NE Atlantic (Rego et al. 1985; Abollo et al. 2001). This result is not surprising considering that *Nyctiphanes couchii* have been described as intermediate host for Anisakids (Smith 1983). Moreover, this species of krill is the main euphausiid in the European continental shelf (Lindley 1977), and is one of the dominant taxa in the mesozooplankton communities found over the shelf during summer and autumn off NW Iberian Peninsula (Roura et al. 2013). Furthermore, this krill is an important prey item of different cephalopods and fish species which are used as paratenic hosts for transmitting *Anisakis simplex* complex (Abollo et al. 1998, 2001; Gestal et al. 1999; Valero et al. 2000; Raga et al. 2009; Roura et al. 2012; Llarena-Reino et al. 2012) towards a definitive host, the marine mammals, by means of predator-prey interactions (Marcogliese 1995).

On the other hand, mysids are the most abundant crustaceans in the hyperbenthos, which are able to undertake vertical migrations and become part of the zooplankton (Jackson et al. 1997). This migration could allow *Anisakis simplex* to overstep habitats creating trophic linkages between benthic and pelagic environments, further increasing the ramifications for their transmission (Marcogliese 2002). In fact, mysids have been underlined to play an important role in the transmission of other anisakid nematodes as *Anisakis simplex*, *Hysterothylacium aduncum*, *Pseudoterranova decipiens*, and *P. adunca* (Køie 1993; Marcogliese 1992, 1993; Jackson et al. 1997; Makings 1981). Furthermore, Køie (1993) reported natural infections in softbodied animals as second intermediate or transport host for anisakids, thus we have considered that salpids were presumably acting in the same way in our study.

Previous records (Karasev 1993; Køie 2001) noticed that in the pelagic habitat it is possible to find free parasitic larvae of anisakid species, which are presumably coming from dead crustacean intermediate host or from dead fish paratenic host. Therefore, the free larvae that we found may have come from the intermediate crustacean host *Nyctiphanes couchii* or from the paratenic hosts such as mysids (Figure 2 M-O), cephalopods, fishes or salpids.

The overall prevalence values of anisakids from mesozooplankton communities off Galicia were low, however it is a common feature of parasites at the zooplankton population level. In spite of the fact that the prevalence is low, most predators ingest large quantities of zooplankton components (euphausiids, mysids, sapids etc.), hence they turn out to be relevant intermediate hosts, which promote high prevalence rates and intensities in higher trophic level organisms as a final parasite's hosts (Marcogliese 1995). Moreover, the prevalence within adult euphausiids was also very low 0.0014%, although their ecological impact is better understood if we consider the whole mesozooplanktonic community where the sample was taken.

Shimazu and Amano (2001) suggested that $L_3$ may be infective for younger euphausiids and/or the euphausiids become infected via copepods as paratenic hosts. In this work, we did not find *Anisakis simplex* $L_3$ infecting the larvae of *Nycthiphanes couchii* (calyptopis and furcilias with 0.8-2.20 and 2.2-5.5 mm in total length, respectively), and neither we did in copepods or other taxa except in mysids and salpids. The absence of $L_3$ in copepods may be influenced by their small size, which is less than 3 mm (total length) for the biggest copopods in the studied area (*Calanus helgolandicus* Claus, 1863, *Calanoides carinatus* Kroyer, 1849 and *Paraeuchaeta hebes* Giesbrecht, 1888). The relative large size of the $L_3$ (~19.03 mm), suggests that only mysids and euphausiids are big enough to host them in their body cavity. Consequently, it seems that in this area the smallest organisms might not harbour large $L_3$ and neither act as intermediate or paratenic host for *A. simplex* $L_3$. Furthermore, none of the examined infected animals showed more than one nematode per individual (Gómez-Gutiérrez et al. 2010), presumably because this intermediate hosts with multi-infections die (Smith 1983; Smith and Mooney 2005).

The current analysis of nematode infection in zooplankton communities provides some insight into the hypothesis that the recruitment of parasites, in these communities, is directly conditioned by the oceanography (Pascual et al. 2007). In fact, under the observed downwelling conditions it seems that the recruitment of *Anisakis* spp. to the mesozooplankton is higher than under upwelling conditions. This argument was also reinforced by other host-parasite systems in the same sampling area (Gregori et al. 2012; Gregori et al. *in press*). If we consider the adults of krill in SF community (185,107), estimations on the potential number of infected individuals by *Anisakis simplex* complex would be 8, while for each anisakid species (*A. simplex s.l.*, *A. simplex s.s.* and *A. pegreffi*) would be 3. On the other hand, krill adults decreased to 64,110 in AF community, as well as the number of plausible infected individuals that would be 3 for *A. simplex* complex, and 1 for each anisakid species *(A. simplex s.l.*, *A. simplex s.s.* and *A. pegreffi).* Regarding salpids, with a prevalence of 0.0073%, the number of potential infected animals declined from 56 in SF to 5 in AF communities. The huge numbers of *Nyctiphanes couchii* adults found in the SF community were owed to a hatching event recorded on July (Roura et al. 2013), coincidently with the reproductive period of this species (Mauchline 1984). This big aggregation of adults, brought us the possibility to find the third-stage larvae of *Anisakis simplex* complex, which coincides with their distribution, mainly in the offshore environment (Abollo et al. 2001), also extending the host range to one hyperbenthic mysid and one salpid (*Salpa fusiformis*) off North the Western Iberian Peninsula.

Overall, this is the first record of *Anisakis pegreffi* in *Nyctiphanes couchii*, which probably acts, through predator-prey interactions, as intermediate host in coastal waters of the NW Iberian Peninsula. As a result, *Anisakis simplex* complex are able to share the same intermediate host in a sympatric distribution among krill. Moreover, it is also the first record of *Anisakis simplex s.l.* in *Salpa fusiformis*, which likely acts as second intermediate or transport hots in this area.

Our results suggest that the recruitment of *Anisakis simplex* complex may be affected by the oceanography and it is different under upwelled or downwelled conditions. The finding of infected mysids and salpids suggested that *Anisakis simplex* complex are not specific at the mesozooplankton level, and indicate that they use different hosts to cross habitats and enlarge the pathways in order to find their definitive mammal host. Additionally, $L_3$ larvae found in the Ría de Vigo, were not homogeneously distributed among mesozooplankton communities.

Further studies should be undertaken to identify the mysid host, as well as to test and evaluate variations of parasite prevalence and recruitment in zooplankton and hyperbenthos under different upwelling/downwelling scenarios.

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Table 1. Records of *Anisakis* larvae in euphausiids and mysids. N = Number of hosts; n = number of infected hosts; % = prevalence

| **Location** | **Hosts** | **N** | **n** | **%** | **Reference** |
|---|---|---|---|---|---|
| | **Euphausiids** | | | | |
| Barents Sea | *Thysanoessa raschii* | ? | 1 | ? | Uspenskaya (1963) |
| Bering Sea and North Pacific Ocean | *T. raschii* | 121 | 3 | 0.020 | Oshima et al. (1969) |
| | *T. longipes* | 405 | 2 | 0.005 | |
| Northern Norh Pacific | *Euphausia pacifica* | 54,000 | 1 | 0.002 | Shimazu et al. (1970) |
| Northeast Atlantic Ocean and Norh Sea | *T. longicaudata* | 950 | 3 | 0.316 | |
| | *Meganyctiphanes norvegica* | 3,178 | 1 | 0.031 | |
| East China Sea | *E. pacifica* | 28,219 | 2 | 0.007 | Kagei (1974) |
| Norh Sea | *T. inermis* | ? | 2 | ? | Lindley (1977) |
| Central northern | *E. krohnii* | | | | |
| Antartic Ocean | *E. vallentini* | 11,233 | 2 | 0.018 | Kagei (1979) |
| East China Sea | *E. nana* | ? | 1 | ? | Shimazu (1982) |
| Northern North Sea. North of Scotland and Faroe | *T. inermis* | | | 0.5-4 | |
| | *T. longicaudata* | | | 0.7-1 | |
| Northern Sea to North of Scotland and At Faroe | *T. inermis* | 1,335 | 8 | 1.3 | |
| | *T. longicaudata* | 335 | 3 | 0,89 | Smith (1971) |
| Northeast Atlantic Ocean and Norh Sea | *T. inermis* | 11,956 | presence | 0-4 | Smith (1983) |
| | *T. longicaudata* | 2,218 | presence | 0-1 | |
| | *T. raschii* | 6,587 | presence | 0-1.3 | |
| | *Nyctiphanes couchii* | 3,067 | presence | ? | |
| South Pacific Ocean | *N. australis* | 11,850 | 3 | 0.0003 | Hurts (1984) |
| St Lawrence Stuary | *T. raschii* | 551,569 | 100 | 0.018 | Hays et al. (1998) |
| | *M. norvegica* | 9,681 | 1 | 0.010 | |
| North West Pacific | *E. pacifica* | | | 0.00002 | Shimazu and Oshima (1972) |
| Prince William Sound. Alaska | *E. pacifica* | 7,447 | | 0.013 | Smith and Mooney (2005) |
| | *T. raschii* | 10,437 | | 0.019 | |
| Northwestern coast of Mexico | *N. simplex* | | | 0.0001 | Gómez-Gutiérrez et al. (2010) |
| Galician Waters | *N. couchii* with *Anisakis pegreffi* | 69,954 | 1 | 0.001 | (Present study) |
| Galician Waters | *N. couchii* with *A. simplex s. s.* | 69,954 | 1 | 0.001 | (Present study) |
| | **Mysids** | | | | |
| Millport W. Scotland | *Mesopodopsis slabberi* | 131 | 1 | 0.763 | Makings 1981 |

Table 2. Composition, number and total abundance of mesozooplankton collected at transect 5 (T5, Figure 1). Shannon-Wiener index for the entire assemblage is shown. SF: Summer Frontal; SO: Summer Ocean; AO: Autumn Ocean communities were $L_3$ were found. N = number of animals; Ab = Abundance N/m$^3$. Filtered volume: 2nd of July column and surface = 410.37 and 685.43 m$^3$ respectively; 4th of July column and surface = 108.51 and 278.72 m$^3$ severaly.

| | **Communities** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **SF** | | | | **SO** | | | |
| **Meroplankton** | **2nd of July column** | | **2nd of July surface** | | **4th of July column** | | **4th of July surface** | |
| | **N** | **Ab** | **N** | **Ab** | **N** | **Ab** | **N** | **Ab** |
| **Cephalopoda** | | | | | | | | |
| Loliginids | | | 1 | 0.00 | | | | |
| *Octopus vulgaris* | | | 1 | 0.00 | | | | |
| Sepiolids | 7 | 0.02 | 7 | 0.01 | | | | |
| **Echinodermata** | | | | | | | | |
| Echinoidea larvae | | | | | | | | |
| Ophiuroidea larvae | 512 | 1.25 | | | | | | |
| **Fish** | | | | | | | | |
| Fish larvae | 512 | 1.25 | 66 | 0.10 | 9 | 0.08 | 50 | 0.18 |
| **Gasteropoda** | | | | | | | | |
| Gastropoda larvae | | | | | 25 | 0.23 | | |
| **Isopoda** | | | | | | | | |
| Aegidae | | | 1 | 0.00 | | | | |
| **Malacostraca** | | | | | | | | |
| **Decapoda** | | | | | | | | |
| Alpheidae zoeae | | | 560 | 0.82 | | | | |
| Brachyura juvenile | | | 82 | 0.12 | 10 | 0.09 | 5 | 0.02 |
| Brachyura megalopa | | | 1,680 | 2.45 | | | 234 | 0.84 |
| Brachyura zoeae | 2,463 | 6.00 | 8,400 | 12.25 | | | | |
| Galatheidae zoeae | | | | | | | | |
| Paguridae megalopa | 480 | 1.17 | 560 | 0.82 | | | | |
| Paguridae zoeae | | | 560 | 0.82 | | | | |
| **Porcellanidae** | | | | | | | | |
| Processidae zoeae | | | 2,844 | 4.19 | | | | |
| *Scyllarus arctus* zoeae | | | | | | | | |
| **Amphipoda Caprellidea** | | | | | | | | |
| **Amphipoda Gammaridea** | 85 | 0.21 | 12 | 0.02 | 3 | 0.03 | 4 | 0.01 |
| **Amphipoda Hyperiidea** | | | | | 11 | 0.10 | 8 | 0.03 |
| **Stomatopoda** | | | | | | | | |
| *Meiosquilla desmaresti* | 480 | 1.17 | | | 123 | 1.13 | | |
| *Platysquilla eusebia* | | | 560 | 0.82 | | | | |
| **Polychaeta** | | | | | | | | |
| Polychaeta larvae | 5 | 0.01 | | | 1 | 0.01 | 7 | 0.03 |

|  | SF |  |  |  | SO |  |  |  |
|---|---|---|---|---|---|---|---|---|
| **Holoplankton** | **2nd of July column** |  | **2nd of July surface** |  | **4th of July column** |  | **4th of July surface** |  |
|  | **N** | **Ab** | **N** | **Ab** | **N** | **Ab** | **N** |  |
| **Appendicularia** |  |  |  |  |  |  |  |  |
| **Chaetognatha** |  |  | 164 | 0.24 | 369 | 3.40 | 1,991 | 7.14 |
| **Cnidaria** |  |  |  |  |  |  |  |  |
| **Hydrozoa** |  |  |  |  |  |  |  |  |
| **Siphonophora** | 1,536 | 3.74 | 8,615 | 12.57 | 542 | 4.99 | 1,206 | 4.33 |
| **Malacostraca** |  |  |  |  |  |  |  |  |
| **Euphausiacea** |  |  |  |  |  |  |  |  |
| *Nyctiphanes couchii* calyptopis | 36,433 | 88.78 | 62,031 | 90.50 | 738 | 6.81 | 10,425 | 37.40 |
| *N. couchii* furcilia | 49,388 | 120.35 | 266,790 | 389.23 | 4,825 | 44.46 | 48,935 | 175.57 |
| *N. couchii* adult | 69,955 | 170.47 | 22,400 | 32.68 | 1,132 | 10.44 | 4,480 | 16.07 |
| **Maxillipoda** |  |  |  |  |  |  |  |  |
| **Copepoda** |  |  |  |  |  |  |  |  |
| **Calanoidea** |  |  |  |  |  |  |  |  |
| *Acartia clausii* | 7,421 | 18.08 | 50,960 | 74.35 | 492 | 4.54 | 2,693 | 9.66 |
| *Candacia armata* |  |  |  |  |  |  |  |  |
| *Calanoides carinatus* | 72,259 | 176.08 | 106,960 | 156.05 | 23,262 | 214.37 | 19,785 | 70.99 |
| *Calanus helgolandicus* | 35,156 | 85.67 | 21,280 | 31.05 | 16,246 | 149.72 | 10,185 | 36.54 |
| *Centropages chierchiae* | 3,966 | 9.67 | 10,080 | 14.71 | 738 | 6.81 | 702 | 2.52 |
| *Clausocalanus spp.* |  |  | 1,120 | 1.63 |  |  | 117 | 0.42 |
| *Mesocalanus tenuicornis* |  |  |  |  |  |  |  |  |
| *Metridia lucens* |  |  |  |  |  |  |  |  |
| *Paracalanus parvus* |  |  |  |  |  |  |  |  |
| *Paraeuchaeta hebes* | 8,637 | 21.05 | 19,040 | 27.78 | 6,031 | 55.58 | 2,810 | 10.08 |
| *Paraeuchaeta sp.* | 4,797 | 11.69 |  |  | 6,646 | 61.25 | 3,512 | 12.60 |
| *Pseudocalanus elongatus* |  |  |  |  |  |  |  |  |
| *Scolecithricella spp.* |  |  | 1,680 | 2.45 |  |  |  |  |
| *Temora longicornis* |  |  |  |  |  |  |  |  |
| Calanoid copepodit |  |  |  |  |  |  |  |  |
| **Mysidacea** | 1,919 | 4.68 | 2,585 | 3.77 | 443 | 4.08 | 1,465 | 5.25 |
| **Thaliacea** |  |  |  |  |  |  |  |  |
| **Salpida** | 21,175 | 51.60 | 6,160 | 8.99 | 5,538 | 51.04 | 28,098 | 100.81 |
| **Total** | 588,036 |  | 866,063 |  | 538,036 |  | 607,565 |  |
| **Indice de Shannon (H')** |  | **1.55** |  | **1.08** |  | **1.81** |  | **1.49** |
| **Eveness index** |  | **0.54** |  | **0.33** |  | **0.63** |  | **0.52** |

Table 2. Continuation. 9th of July column and surface = 830.01 and 193.26 m$^3$ respectively; 26th of September column and surface = 220.78 and 34.11 m$^3$ severaly.

| **Communities** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **SO** | | **SF** | | **AO** | | | |
| **Meroplankton** | **9th of July column** | | **9th of July surface** | | **26th of September column** | | **26th of September surface** | |
| | **N** | **Ab** | **N** | **Ab** | **N** | **Ab** | **N** | **Ab** |
| **Cephalopoda** | | | | | | | | |
| Loliginids | | | | | 2 | 0.01 | | |
| *Octopus vulgaris* | 3 | 0.00 | | | 1 | 0.00 | | |
| Sepiolids | 1 | 0.00 | 1 | 0.01 | | | | |
| **Echinodermata** | | | | | | | | |
| Echinoidea larvae | | | 800 | 4.14 | | | | |
| Ophiuroidea larvae | | | | | | | | |
| **Fish** | | | | | | | | |
| Fish larvae | 73 | 0.09 | 307 | 1.59 | 29 | 0.13 | 2 | 0.06 |
| **Gasteropoda** | | | | | | | | |
| Gastropoda larvae | | | | | | | | |
| **Isopoda** | | | | | | | | |
| Aegidae | | | | | | | | |
| **Malacostraca** | | | | | | | | |
| **Decapoda** | | | | | | | | |
| Alpheidae zoeae | 267 | 0.32 | 267 | 1.38 | | | | |
| Brachyura juvenile | 4 | 0.00 | | | 2 | 0.01 | | |
| Brachyura megalopa | | | | | | | 8 | 0.22 |
| Brachyura zoeae | 11,467 | 13.82 | | | | | | |
| Galatheidae zoeae | | | | | | | 8 | 0.22 |
| Paguridae megalopa | | | | | | | | |
| Paguridae zoeae | | | | | | | | |
| **Porcellanidae** | | | | | | | | |
| Processidae zoeae | | | | | | | | |
| *Scyllarus arctus* zoeae | 267 | 0.32 | | | | | | |
| **Amphipoda Caprellidea** | | | | | | | 1 | 0.03 |
| **Amphipoda Gammaridea** | 30 | 0.04 | 8 | 0.04 | 6 | 0.03 | | |
| **Amphipoda Hyperiidea** | 12 | 0.01 | 4 | 0.02 | 5 | 0.02 | | |
| **Stomatopoda** | | | | | | | | |
| *Meiosquilla desmaresti* | | | | | | | | |
| *Platysquilla eusebia* | | | | | | | | |
| **Polychaeta** | | | | | | | | |
| Polychaeta larvae | 24 | 0.03 | | | 35 | 0.16 | | |

|  | **SO** |  | **SF** |  | **AO** |  |  |  |
|---|---|---|---|---|---|---|---|---|
| **Holoplankton** | **9th of July column** |  | **9th of July surface** |  | **26th of September column** |  | **26th of September surface** |  |
|  | **N** | **Ab** | **N** | **Ab** | **N** | **Ab** | **N** | **Ab** |
| **Appendicularia** |  |  |  |  |  |  |  |  |
| **Chaetognatha** | 5,408 | 6.52 | 2,933 | 15.18 | 1,861 | 8.43 | 1,558 | 45.69 |
| **Cnidaria** |  |  |  |  |  |  | 30 | 0.89 |
| **Hydrozoa** |  |  |  |  |  |  |  |  |
| **Siphonophora** | 2,031 | 2.45 | 1,692 | 8.76 | 107 | 0.48 | 15 | 0.44 |
| **Malacostraca** |  |  |  |  |  |  |  |  |
| **Euphausiacea** |  |  |  |  |  |  |  |  |
| *Nyctiphanes couchii* calyptopis | 6,277 | 7.56 | 15,385 | 79.60 | 2,496 | 11.31 | 83 | 2.43 |
| *N. couchii* furcilia | 1,385 | 1.67 | 2,000 | 10.35 | 4,512 | 20.44 | 8 | 0.22 |
| *N. couchii* adult | 1,292 | 1.56 | 154 | 0.80 | 3,600 | 16.31 | 98 | 2.88 |
| **Maxillipoda** |  |  |  |  |  |  |  |  |
| **Copepoda** |  |  |  |  |  |  |  |  |
| **Calanoidea** |  |  |  |  |  |  |  |  |
| *Acartia clausii* | 19,733 | 23.77 | 17,867 | 92.45 | 1,263 | 5.72 | 1,072 | 31.42 |
| *Candacia armata* | 267 | 0.32 |  |  |  |  |  |  |
| *Calanoides carinatus* | 50,933 | 61.36 | 43,733 | 226.29 | 1,600 | 7.25 |  |  |
| *Calanus helgolandicus* | 22,133 | 26.67 | 7,467 | 38.63 | 1,853 | 8.39 | 53 | 1.55 |
| *Centropages chierchiae* | 1,067 | 1.29 | 267 | 1.38 |  |  |  |  |
| *Clausocalanus spp.* | 267 | 0.32 | 533 | 2.76 |  |  | 8 | 0.22 |
| *Mesocalanus tenuicornis* |  |  |  |  | 253 | 1.14 | 15 | 0.44 |
| *Metridia lucens* | 1,333 | 1.61 | 267 | 1.38 | 1,432 | 6.48 | 23 | 0.66 |
| *Paracalanus parvus* | 267 | 0.32 |  |  | 253 | 1.14 | 30 | 0.89 |
| *Paraeuchaeta hebes* | 7,467 | 9.00 | 7,200 | 37.25 | 7,326 | 33.18 | 513 | 15.05 |
| *Paraeuchaeta sp.* | 30,667 | 36.95 | 29,600 | 153.16 | 20,463 | 92.69 | 732 | 21.46 |
| *Pseudocalanus elongatus* |  |  | 800 | 4.14 |  |  |  |  |
| *Scolecithricella spp.* |  |  |  |  |  |  |  |  |
| *Temora longicornis* |  |  |  |  |  |  | 8 | 0.22 |
| Calanoid copepodit |  |  | 267 | 1.38 |  |  |  |  |
| **Mysidacea** | 831 | 1.00 | 2,769 | 14.33 | 528 | 2.39 | 211 | 6.20 |
| **Thaliacea** |  |  |  |  |  |  |  |  |
| **Salpida** | 11,467 | 13.82 | 22,133 | 114.52 | 3,874 | 17.55 | 1,389 | 40.72 |
| **Total** | 1,145,821 |  | 1,127,305 |  | 2,742,356 |  | 2,696,716 |  |
| **Shannon index (H')** |  | **2.12** |  | **2.09** |  | **1.62** |  | **1.89** |
| **Eveness index** |  | **0.66** |  | **0.68** |  | **0.54** |  | **0.64** |

Table 3. $L_3$ of the *Anisakis simplex* complex found on each mesozooplankton community (C). IK: number of infected krill; P.% *N. couchii* [CI]: Population prevalence of *N. couchii* and confidence interval; A. % *N. couchii* [CI]: Adults prevalence of *N. couchii* and confidence interval; IS: number of infected Salpidae; S. % [CI]: Salpidae prevalence and confidence interval; IM: Number of infected Mysidae; AbM: Abundance of $L_3$ among Mysidae: WC: Number of $L_3$ found free on the water column; AbW: Abundance ($L_3/m^3$).

| **C** | | **Parasite species** | **IK** | **P.% *N. couchii* [CI]** | **A. % *N. couchii* [CI]** | **IS** | **S. % [CI]** | **IM** | **AbM** | **WC** | **AbW** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Summer Frontal** | 2nd of July column | *Anisakis simplex s.l.* | 1 | 0.00064<br>[0,00002 – 0.00094] | 0.00143<br>[0.00004 – 0.00796] | 1 | 0.00472<br>[0.00012 - 0.0070] | | | 2 | 0,005 |
| | | *Anisakis simplex* s.s | 1 | 0.00064<br>[0.00002 - 0.00094] | 0.00143<br>[0.00004 - 0.00796] | | | | | 3 | 0,007 |
| | | *Anisakis pegreffi* | 1 | 0.00064<br>[0.00002 - 0.00094] | 0.00143<br>[0.00004 - 0.00796] | | | | | 4 | 0,01 |
| | 2nd of July surface | *Anisakis simplex* s.l. | | | | 1 | 0.01623<br>[0.00041 - 0.0239] | | | | |
| | Total | *Anisakis simplex* complex | 3 | 0.00192<br>[0.004 – 0.0018] | 0.00428<br>[0.00088 – 0.0125] | 2 | 0.00732<br>[0.00887 – 0.0078] | | | | |
| **Summer Oceanic** | 4th of July column | *Anisakis simplex s.s.* | | | | | | | | 1 | 0,009 |
| | 4th of July surface | *Anisakis simplex* s.l. | | | | | | | | 1 | 0.003 |
| | 9th of July column | *Anisakis simplex* s.l. | | | | | | 1 | 0.120 | | |
| **Autumn Oceanic** | 26th of September column | *Anisakis simplex s.s.* | | | | | | | | 1 | 0.004 |

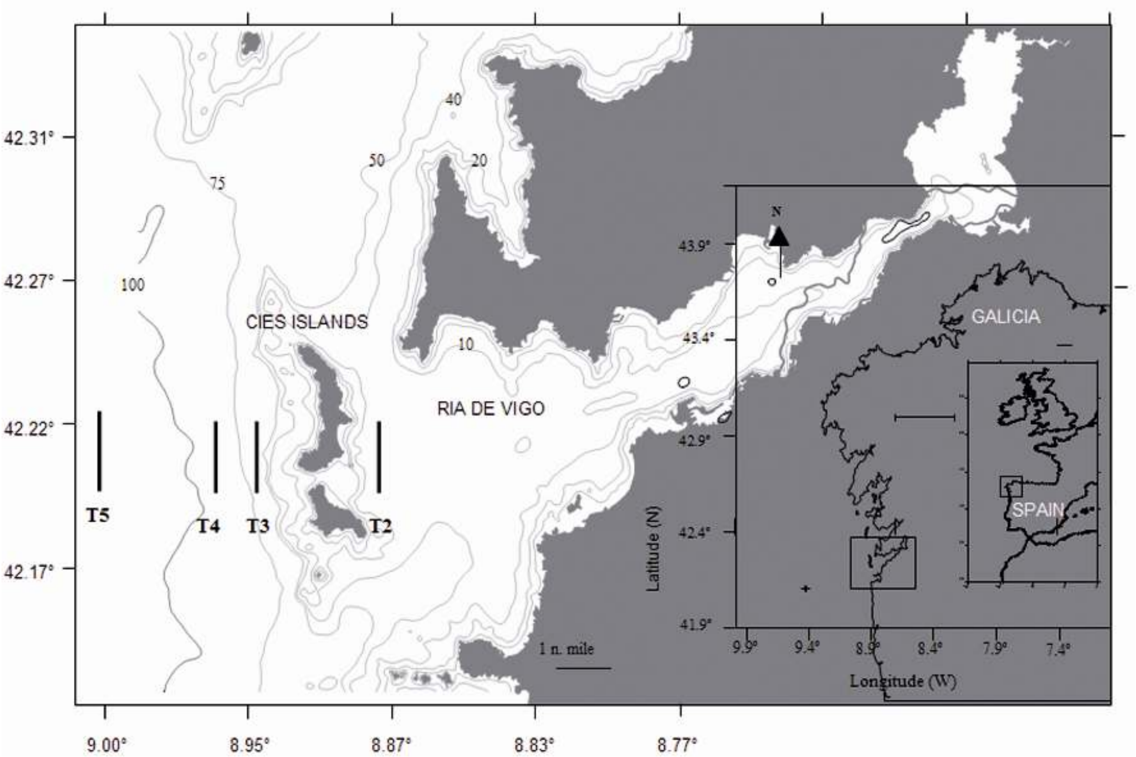

Figure 1. Sampling area. Ría de Vigo in Galician waters, NW Iberian Peninsula. T5 = Transect 5, T4 = Transect 4, T3= Transect 3 and T2 = Transect 2.

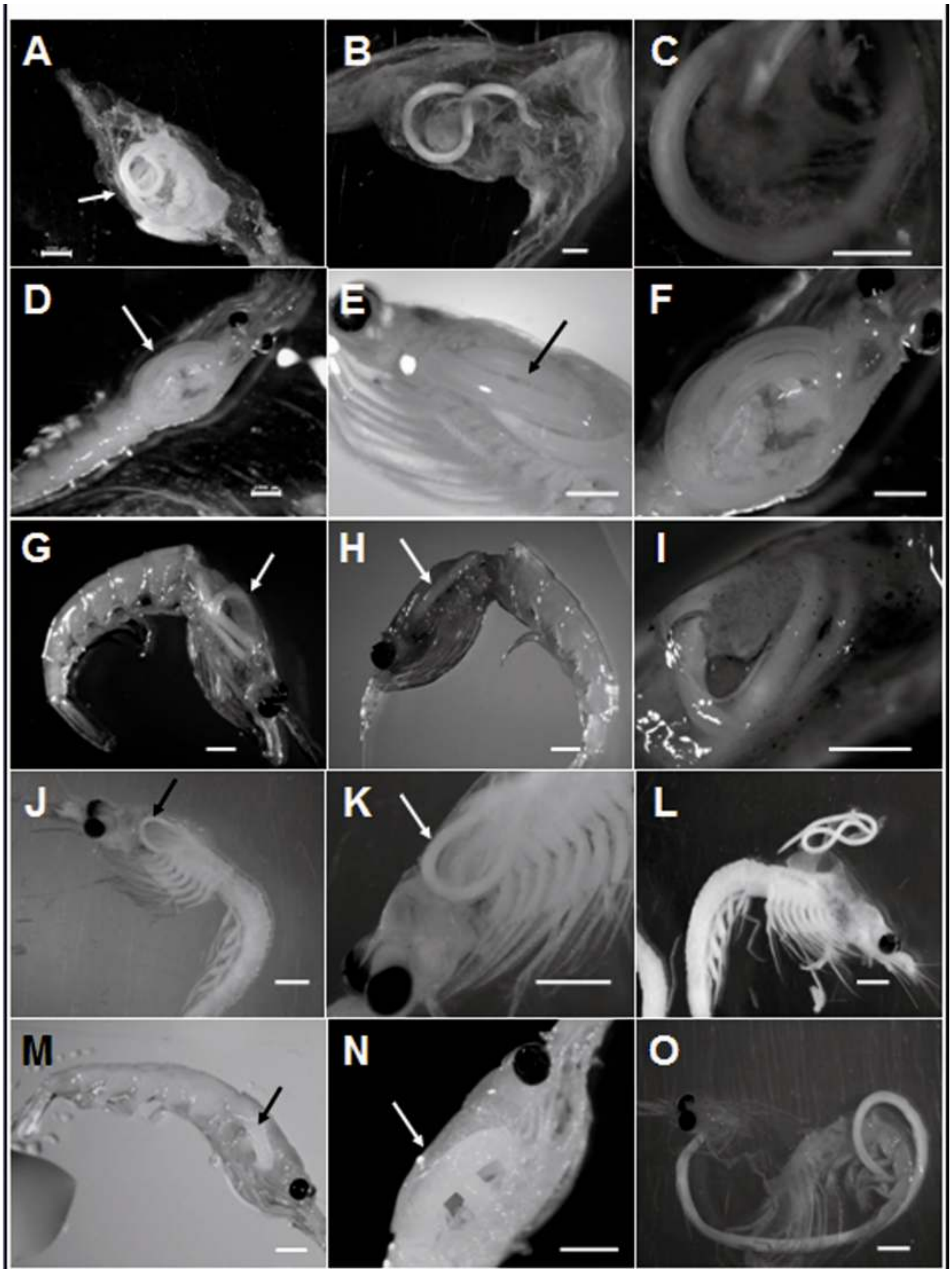

Figure 2. (A-C) *Anisakis simplex s.l.* third-stage larvae ($L_3$) from *Salpa fusiformis*. (D-F) Single infection with one *Anisakis simplex s.s.* third-stage larvae ($L_3$) from *Nyctiphanes couchii*. (G-I) Single infection with *Anisakis pegreffi* third-stage larvae ($L_3$) from *Nyctiphanes couchii*. (J-L) Single infection with *Anisakis simplex s.l.* third-stage larvae ($L_3$) from Mysidae. (M-O) *Anisakis simplex s.l.* emerging from a *Nyctiphanes couchii*.