Deep-Sea Research I 104 (2015) 106–121

Contents lists available at ScienceDirect

Deep-Sea Research I

journal homepage: www.elsevier.com/locate/dsri

Annual variation of parasite communities of deep-sea macrourid fishes from the western Mediterranean Sea and their relationship with fish diet and histopathological alterations

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ARTICLE INFO

Article history:
Received 16 March 2015
Received in revised form
3 July 2015
Accepted 4 July 2015
Available online 9 July 2015

Keywords:
Hymenocephalus italicus
Nezumia aequalis
Trachyrincus scabrus
Western Mediterranean
Parasites
Pathology
Diet

ABSTRACT

Parasite communities of three abundant benthopelagic macrourid species (*Hymenocephalus italicus*, *Nezumia aequalis* and *Trachyrincus scabrus*) of the upper slope from the western Mediterranean were analysed seasonally. Histopathological, dietary and environmental information (temperature, salinity, O_2 and turbidity) were also obtained. The three fish hosts shared only three parasite species (the nematodes *Raphidascaris macrouri* and *Hysterothylacium aduncum* and the acanthocephalan *Echinorhynchus trachyrinci*). *H. italicus*, the most benthopelagic fish, showed low parasite richness and diversity. The highest total mean abundance of parasites was found in spring for *H. italicus* and *T. scabrus*, coinciding with the highest prevalence/abundance of the majority of parasites whereas parasites of *N. aequalis* exhibited the highest richness, mean abundance and diversity in winter. Parasites related with benthic or infaunal preys were linked to autumn and summer samples off Besós (Barcelona). Some parasites were also linked to high turbidity, which may be due to higher abundances of the intermediate hosts, such as near-bottom zooplaktonic or suprabenthic preys. Few histopathological alterations (e.g. cysts of unknown aetiology) were observed restricted to the two most benthic-feeding fish species inhabiting more closely the near-bottom/sediment level, especially in autumn.

1. Introduction

The Macrouridae are one of the most dominant deep-water demersal fish families with approximately 300 species, 90% of which live in the continental slope between 200 and 2000 m depth (Marshall, 1965; Cohen et al., 1990). Eight species of macrourids occur in the western Mediterranean Sea (Stefanescu et al., 1993) but only five are common on the upper slope off the Catalonian coasts of Spain (Cartes et al., 2009; Papiol et al., 2012; Fanelli et al., 2013): *Coelorinchus caelorhincus* (Risso, 1810) (hollowsnout grenadier), *Coelorinchus labiatus* (Köhler, 1896) (spearsnouted grenadier), *Hymenocephalus italicus* Giglioli, 1884 (glasshead grenadier), *Nezumia aequalis* (Günther, 1878) (common Atlantic grenadier) and *Trachyrincus scabrus* (Rafinesque, 1810) (roughsnout grenadier). Of these common species, *N. aequalis*, and *T. scabrus* are the most abundant between depths of 600–800 m (Papiol et al., 2012; Fanelli et al., 2013). These species constitute a large proportion of the by-catch in fisheries (Moranta et al., 2000) and play an important role in the bathyal food web and the deep-sea megafauna (Geistdoerfer, 1978; Macpherson, 1979; Merrett and Haedrich, 1997). *N. aequalis* is widely distributed throughout the Atlantic Ocean and Mediterranean Sea (Cohen et al., 1990). In the Mediterranean, its highest abundances exhibit a bimodal distribution in relation to depth (between 700 and 800 m and between 1000 and 1100 m) (Massutí et al. 1995). It has a benthic diet (Fanelli and Cartes, 2010), mainly feeding on crustaceans (mostly amphipods) and polychaetes (Carrassón and Matallanas, 1989; Madurell and Cartes, 2006). *T. scabrus* is the most abundant macrourid species between 900 and 1000 m (Massutí et al. 1995). Its diet also consists of benthic and bentho-pelagic organisms such as copepods, amphipods and polychaetes (Macpherson, 1979; Merrett and Marshall, 1981; Carrassón and Matallanas, 2002), but this species displays a particular preference for bathypelagic preys

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http://dx.doi.org/10.1016/j.dsr.2015.07.002
0967-0637/

(calanoid copepods and mysids) (Carrassón and Matallanas, 2002). *H. italicus* is common at depths of 400–600 and is less abundant at lower depths until disappearing around 900 m (Massutí et al., 1995). The ecology of this specie is different to the other macrourids studied in the Mediterranean because of its high swimming capacity and benthopelagic habitats (Marshall and Merret, 1977; McLellan, 1977; Geistdoerfer, 1978; Madurell and Cartes, 2006; Fanelli and Cartes, 2010). Its diet comprises euphausiids, sergestids and other migrant crustaceans living in the benthic boundary layer (Marshall and Merret, 1977; McLellan, 1977; Geistdoerfer, 1978; Macpherson, 1979; Madurell and Cartes, 2006; Fanelli and Cartes, 2010).

Despite the broad range of issues assessed in relation to these grenadier species, the information on their parasite fauna is scarce in the Mediterranean Sea. In general, parasites of deep-sea fish are often neglected, even though they are important components of all marine environments (Klimpel et al., 2001; Palm et al., 1999) and an integral part of the deep-sea ecosystems (Klimpel et al., 2006). Bray (1995) surveyed the occurrence of digenea in macrourid fishes and in a global survey of deep-sea fishes showed that only four digenean families reach abyssal depths greater than 4000 m, parasite species diversity generally decreasing with depth (Bray, 2004). The study of the parasites can provide relevant ecological and biological information about their hosts (Campbell et al., 1980; Palm et al., 1998), for example on the trophic status within the marine ecosystem (Klimpel et al., 2003). In particular, the heteroxenous parasites reflect the trophic relationships between the aquatic animals that are infected by the different stages of their life-cycles, since they pass through the marine food web to reach the definitive host (Campbell et al., 1980). In addition, parasites are increasingly being used as natural biological tags in multidisciplinary studies on effects of pollution and environmental stress in aquatic ecosystems (MacKenzie et al., 1995; MacKenzie, 2002; Williams and MacKenzie, 2003; Marcogliese, 2005; Pérez-del-Olmo et al., 2007; Vidal-Martínez et al., 2010;). Furthermore, parasite infections may result pathologies in different organs and tissues of the fish hosts thus reducing their survival chances. These host–parasite interactions can change, from the initial penetration of host cells, ingestion or attachment to establishment, growth, multiplication and senescence (Feist and Longshaw, 2008). Therefore, histopathology may provide good data and indicators of prognostic value in parasitic studies.

Some parasites were described in *H. italicus*, *N. aequalis* and *T. scabrus* from the Atlantic Ocean (Bray, 1995; Klimpel et al., 2001): digeneans have been reported from the three fish species, monogeneans from *N. aequalis* and the acanthocephalan *Echinorhynchus trachyrinci* from *T. scabrus*. There are also some parasitological studies on other macrourid species (such as *Coryphaenoides rupestris* Gunnerus, 1765 and *C. mediterraneus* (Giglioli, 1893), *Macrourus berglax* Lacepède, 1801 , *Nezumia bairdii* (Goode and Bean, 1877) and *Nezumia pulchella* (Pequeño, 1971)) in the Atlantic Ocean (Zubchenko, 1981; Klimpel et al., 2001; Kellermanns et al., 2009; Palm and Klimpel, 2008), Pacific Ocean (Salinas et al. 2008) and Arctic Sea (Klimpel et al., 2006). However, the knowledge on the parasite fauna of *H. italicus*, *N. aequalis* and *T. scabrus* in the Mediterranean Sea is almost inexistent, since so far just a single digenean, *Bathycreadium brayi* (see Pérez-del-Olmo et al., 2014) (syn. *Bathycreadium elongatum* of Constenla et al. (2011)), has been described in *T. scabrus* from the western Mediterranean basin. Knowledge of the parasite fauna of the Mediterranean macrourids is of special interest because they (as all members of the deep-sea fauna, Pérès, 1985) exhibit low species richness and thus offer few potential hosts to parasites.

The aim of the present study is to provide, for the first time, detailed information on the seasonal variability of the parasite communities from three dominant macrourid species (*H. italicus*, *N. aequalis* and *T. scabrus*) of the upper slope in the western Mediterranean Sea, in relation to environmental parameters and prey ingested. General fish condition and fish potential pathologies are also explored in order to test for possible relationships with the parasite load.

2. Materials and methods

2.1. Sampling area and parasite collection

Total of 92 specimens of *H. italicus*, 115 of *N. aequalis,* and 171 of *T. scabrus* were captured in 2007 and 2008 (Table 1) from the continental slope off Barcelona (central coast of Catalonia, northeastern Spain), at Besòs Canyon and its adjacent slope and also to the South, off Vilanova slope, at depths between 600 and 800 m (Table 1). Samples were collected by the R/V "García del Cid" with a semi-balloon otter-trawl (OTSB), and with a commercial fishing gear (bou), within the framework of the MEC Spanish oceanographic projects BIOMARE (CTM2006-13508-C02-01/02/MAR) and ANTROMARE (CTM2009-12214-C02-01/02). Sampling was carried out seasonally (Table 1). Environmental data (temperature (*T*) in ºC, salinity (S) in PSU, O_2 concentration in ml/l and turbidity (voltage)) were taken at 5 m above the sea-bottom by deployment of a CTD almost simultaneously (same data, and close in time) to hauls performed to sample fish specimens.

Fish were processed on board immediately after capture. Preanal length (PAL) of each fish was measured to the nearest millimetre and body weight was taken to the nearest gram. Captured specimens were processed in different ways: (i) 41 *H. italicus*, 42 *N. aequalis* and 45 *T. scabrus* were processed for both parasitological and histological studies: entire liver and spleen and left hemibranch of each specimen were fixed in 10% buffered formalin for histopathological studies, and the rest of the specimen was frozen (−20 °C) for a subsequent parasitological study; (ii) 39 specimens of *H. italicus*, 49 of *N. aequalis* and 42 of *T. scabrus* were frozen (−20 °C) immediately after capture and were added to the parasitological study; and (iii) 12 specimens of *H. italicus*, 24 of *N. aequalis*, and 84 of *T. scabrus* were fixed "*in toto*" in 10% buffered formalin with the abdominal cavity opened to help the fixation and preservation of internal organs and were added to the histopathological study. Liver and gonads of each specimen were also weighed to the nearest gram. Fishes and organs fixed in 10% buffered formalin were dissected and entire liver and spleen, left and right gill hemibranches and entire digestive tract (only for specimens sampled in summer off Besòs) were processed by routine paraffin histology techniques. Sections (4 μm) of each organ were stained with Haematoxylin and Eosin. Frozen fish were thawed, dissected and examined under a stereomicroscope for the presence of parasites. Parasites from thawed specimens were collected, counted and preserved in 70% ethanol. Digeneans and acantocephalans were stained with iron acetic carmine, dehydrated through a graded ethanol series, cleared in dimethyl phthalate, and mounted in Canada balsam. Nematodes were cleared in glycerine before identification. Parasites were identified to genus or species level whenever possible.

Two nematode larvae of the genus *Hysterothylacium* (one of *N. aequalis* and the second of *T. scabrus*) were used for molecular analyses. DNA from all samples was extracted with Qiagen TM (Valencia, California) DNeasy® Blood and Tissue Kit. The ITS region was amplified by PCR using the primers A (forward: 5-GTCGAATTCGTAGGTGAACCTGCG GAAGGATCA-3) and B (reverse: 5-GCCGGATCC GAATCCTGGTTAGTTTCTTTTCCT-3) (D'Amelio et al., 2000) at 25 μM in 50 μL PCR reaction volume. PCR was performed under the following conditions: initial denaturation of 94 °C for 5 min, followed by 30 amplification cycles of 94 °C for