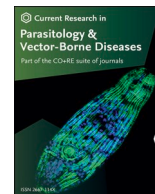




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A case of abnormal swimming patterns in juvenile *Oblada melanura* naturally infected with *Philometra obladae* (Nematoda: Philometridae) in the Tyrrhenian Sea off Sicily, Italy

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ABSTRACT

The saddled seabream, *Oblada melanura* (L.), is a common seawater species present in the Mediterranean. Between July and August 2023, during diving activities along the Sicilian coast of Italy, we recorded with a digital camera several specimens of *O. melanura* showing an abnormal pattern of swimming in the water column. The unusual swimming behaviour was characterized by fast, uncoordinated directional changes and isolation from the remaining fishes in the shoal. Four dead fish were found and collected for necropsy and parasitological evaluation/examination. Upon gross examination, all fish showed an evident coelomic distension; the coelomic cavity of two fish was filled with nematodes that dislocated the coelomic organs, and the other two had degraded nematodes and a conspicuous quantity of fluid in the coelomic cavity. All collected parasites were identified as *Philometra obladae* (Nematoda: Philometridae) according to morphological criteria. Here, we describe the unusual swimming behaviour of *O. melanura* naturally infected with *Ph. obladae* and the results of the examination of dead fish infected with this parasite. Future studies are needed to better evaluate and describe the dynamics and the epidemiology of *Ph. obladae* infection in wild *O. melanura*.

1. Introduction

Saddled seabream *Oblada melanura* (L.) is a seawater species belonging to the family Sparidae. *Oblada melanura* is a common opportunistic predator, present in the Central Mediterranean Sea (Jaridas, 1996), as well as the Eastern Atlantic (Bay of Biscay, the Mediterranean, and from the Strait of Gibraltar to Angola), also known from Madeira, Cape Verde and the Canary Islands, such as along the Egyptian coasts (Mahmoud, 2010). This species inhabits littoral waters, mainly between rocky bottoms and *Posidonia* beds (Bauchot and Hureau, 1986) and is an economically important commercial species, highly appreciated by Mediterranean consumers (Froese and Pauly, 2015).

Nematodes belonging to the genus *Philometra* Costa, 1845 include a large number of species parasitizing many freshwater, seawater and brackish-water teleosts worldwide. Female specimens are bigger than males, from a few centimetres up to 1 m in total length, while males are usually between 2 and 4 mm in length (Moravec, 2004).

Adult nematodes belonging to the genus *Philometra* penetrate the host coelomic cavity through the intestine wall, migrating and consequently inhabiting the swim bladder serosa, where both male and female

larvae develop to a sexually mature stage before mating (Molnár, 1967). Immature female specimens continue their development, reaching the adult stage in the host body cavity until the uterus is filled with free first-stage larvae, up to the end of the following spring or early summer (Moravec, 1977). In Europe, between spring and summer, from late May to the end of June (Molnár, 1967), females of *Philometra* spp. leave the body cavity of the host through tissues, in the proximity of the anus of the host. A hypotonic effect causes the immediate release of the free first-stage larvae, i.e. due to the osmotic differences of the surrounding water (Molnár, 1967; Moravec, 1977). In the case of severe infection, these parasites can be considered highly pathogenic, due to, above all, the large size of the female specimens, mainly localized in the fish ovary, causing severe damage to the coelomic organs, and consequently influencing fish reproduction (Moravec et al., 1997, 2002). Studies on infections with *Philometra* spp. carried out in the Mediterranean resulted in the descriptions of two new species: *Philometra obladae* Moravec, Gaglio, Panebianco & Giannetto, 2008 based on a single gravid female found in the coelomic cavity of *O. melanura* collected in the Tyrrhenian Sea off Sicily, Italy (Moravec et al., 2008a); and *Philometra spicariae* Moravec, Gaglio, Giannetto & Marino, 2010 based on a single gravid

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female found in the coelomic cavity of the picarel *Spicara smaris* (L.) collected in the Ionian Sea off Sicily, Italy (Moravec et al., 2010). Further, one gravid female of *Philometra filiformis* (Stossich, 1896) was described in the ovary of *P. erythrinus* sampled in the Tyrrhenian Sea off Sicily, Italy (Moravec et al., 2008a).

The presence of nematodes of the genus *Philometra* globally, was reported by São Clemente et al. (2017) who morphologically and molecularly characterized *Philometra saltatrix* Ramachandran, 1973 in the gonads of bluefish *Pomatomus saltatrix* (L.) collected off the Mediterranean coast of Tunisia, thus confirming the previous records (Moravec et al., 2008b; Moravec and de Buron, 2009).

Regarding other host species in the Mediterranean, one of the commonest fish species parasitized by nematodes belonging to the genus *Philometra* is the dusky grouper *Epinephelus marginatus* (Lowe) infected with *Philometra jordanoi* López-Neyra, 1951. A male specimen embedded in the ovary of *E. marginatus* collected off Majorca Island, Spain (Western Mediterranean) was morphologically described by Merella et al. (2005). Female specimens of *Ph. jordanoi* have been reported recently in the ovary of four *E. marginatus* sampled off Sicily, Italy (De Benedetto et al., 2021).

Here, we report the unusual swimming behaviour of juvenile specimens of *O. melanura* naturally infected with *Ph. obladae* and the alterations observed in dead fish infected with this hematophagous parasite.

2. Materials and methods

2.1. Video description and fish recovered

In July 2023, during diving off the coast of the Capo D'Orlando municipality, Messina Province, Tyrrhenian Sea (38°09'25.1"N, 14°45'38.0"E), some *O. melanura* specimens were observed to show abnormal behaviour patterns, swimming in the water column. At the same time, two specimens of the same species were found dead on the sea bottom. Due to this uncommon situation, monthly monitoring evaluation from July to October was carried out and video recording of the fish behaviour was performed using a GoPro Hero7 digital camera (GoPro Inc., San Mateo, USA).

2.2. Parasitological examination

The dead fish specimens were sampled, photographed, stored at 4 °C and transferred to the Laboratory of Parasitology and Parasitic Disease of the Department of Veterinary Sciences, University of Messina, Italy, for necropsy and parasitological examination. Fishes were assigned individual consecutive numbers, measured (total body length; accuracy of 0.1 cm) with a ruler and weighed (total body weight) with a digital scale (SW-260548, Baxtram, Girona, Spain; accuracy of 0.5 g); fish age was calculated according to Burak Daban et al. (2020). Coelomic cavities of the specimens were inspected to detect any parasites. All retrieved nematodes were isolated in a Petri dish under a stereomicroscope (Stereo Discovery-V12, Zeiss, Jena, Germany), washed twice in a saline solution and then fixed in 70% ethanol before identification.

For morphological assessment, nematodes were cleared in glycerine for 24 h, mounted on temporary slides in glycerine and examined under a light microscope (Axioskop2, Zeiss, Jena, Germany). Measurements are presented as the range followed by the mean and standard deviation in parentheses. The nematodes were identified to the species level using the key and data in Moravec et al. (2008a). Pictures of mounted parasites were taken using a digital system (Axiocam-Mrc/Axiovision, Zeiss, Jena, Germany).

3. Results

3.1. Unusual swimming behaviour of parasitized fish

Parasitized fish with evident coelomic cavity distension showed

unusual swimming behaviour, characterized by fast, uncoordinated direction changes, isolation and reduction of movement. The fish also showed no reluctance to contact with operator (Video S1).

Supplementary video related to this article can be found at <https://doi.org/10.1016/j.crpvbd.2023.100167>

In other cases, some fish appeared isolated from other specimens of the same shoal. Moreover, up to three parasitized fish swimming together were recorded, isolated from others, also showing abnormal swimming behaviour. The parasitized fish appeared to make up more than 50% of the fish stock present in the examined area (Video S2).

Supplementary video related to this article can be found at <https://doi.org/10.1016/j.crpvbd.2023.100167>

Furthermore, considering the presence of the operator, fish did not show any reluctance to contact with humans, clearly highlighting some differences with healthy fish: in some cases, after repeated stimulations, some specimens showed sudden escape movements (Video S3).

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During the study period, two dead fish were found in July and two other dead specimens of a similar size were found in August. During September and October, none of the observed fish showed abdominal distension or abnormal swimming behaviour.

3.2. Parasitological examination

The four dead *O. melanura* specimens examined weighted 11–18 (14.3 ± 3.1) g and had a total length of 8.5–11.5 (10.4 ± 1.3) cm; the age of all specimens was estimated as < 1 year. The coelomic cavity distension was attributable to the presence of nematodes in two of the four parasitized *O. melanura* found in July, which also showed an abnormal whitish gill colouration (Fig. 1A), completely superimposable with anaemia. Examination of the coelomic cavity showed total



Fig. 1. *Oblada melanura* collected off Capo D'Orlando municipality. **A** Fish with whitish gills, macroscopic evaluation of anaemic status (arrow). **B** Juvenile fish with coelomic cavity completely filled with gravid specimens of *Philometra obladae* (arrows).

dislocation of the internal organs, due to the presence of five gravid female nematodes of the family Philometridae (Fig. 1B).

The nematodes measured 170–202 (186 ± 16) mm in length and 1.13–1.21 (1.17 ± 0.4) mm in width at the cephalic region and 1.12–1.16 (1.14 ± 0.2) mm in width at the caudal region. The oesophagus (including the anterior bulbous inflation) is 1.5–2.1 (1.8 ± 0.3) mm long and 133–187 (165 ± 22) μm wide. The light brown intestine ends blindly in the caudal region, where it appears thinner than in the vulval area. The uterus is strongly developed, between the cephalic and caudal region, reaching anteriorly the anterior level of oesophagus, filled with eggs at different developmental stages, 29–56 (41 ± 7) μm in width, and larvae; the uterine wall was not clearly visible. Vulva, vagina and anus were not observed.

In the other two fish specimens found dead in August, the coelomic cavity was not filled with whole parasites, and showed normal topography of the internal organs. However, degraded nematodes were extracted in the ventral part of the cavity; in these cases, a conspicuous quantity of fluid was observed and sampled. Fluid examination showed the presence of some degraded female nematodes (Fig. 2A) and numerous larvae characterized by a filiform body 550–600 (572 ± 23) μm in length (Fig. 2B). The width of the cephalic and caudal regions of the larvae are 8–9 (8 ± 1) μm and 6–7 (6 ± 0.4) μm , respectively, and the oesophagus is 164–179 (174 ± 7) μm long. Morphological evaluation of all parasitic stages isolated, compared to the data in the key and the original description by Moravec et al. (2008a), allowed the identification of both adults and larvae as *Philometra obladae*.

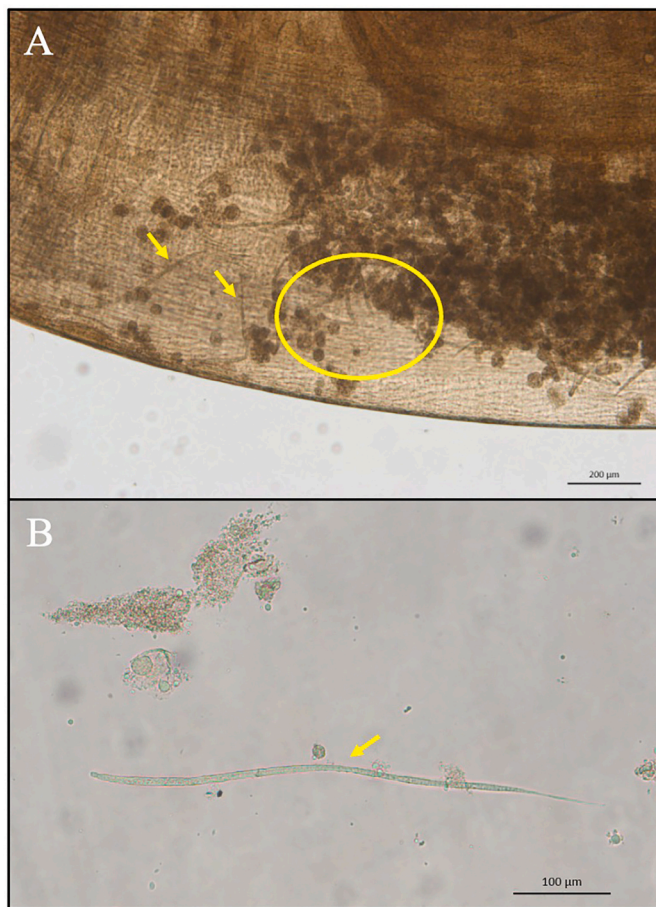


Fig. 2. *Philometra obladae* collected from the coelomic cavity of *Oblada melanura*. A Uterus of *Ph. obladae* after clearing, showing first-stage larvae (arrows) and eggs at different developmental stages (circle). B Free first-stage larvae of *Ph. obladae* after clearing (arrow).

4. Discussion

The present survey represents the first description of unusual behaviour of wild fish infected by nematodes belonging to the genus *Philometra* in the central Mediterranean Sea. In our study, abnormal coelomic distension, as well as uncommon swimming behaviour associated to *Philometra obladae* infection, has been described, also providing some video description of these findings. In agreement with previous studies (Moravec et al., 2003, 2008a,b, 2010; Merella et al., 2004, 2005; De Benedetto et al., 2021), the present survey highlights the importance of the Mediterranean basin for future epidemiological studies on nematodes belonging to the genus *Philometra*. Considering the hematophagous aspect of these nematodes (de Buron and Roumillat, 2010), the infection reported in the present study, compared to the small fish size, could be considered the reason for the gill anaemia observed in parasitized fish.

A study conducted by Séguin et al. (2011) reported unusually high rate of mortality and histopathological and anamnestic description, as well as abdominal distension in captive-raised fingerlings of the striped bass *Morone saxatilis* (Walbaum) originally collected from the River Miramichi, New Brunswick, Canada, due to infection with *Philometra* sp. The genus *Philometra* includes a large number of species, each of them characterized by a peculiar tropism (Moravec et al., 2017). Thus, e.g. *Ph. jordanoi* has been found in the gonads of *E. marginatus* (Moravec et al., 2016), whereas *Ph. obladae* did not show any peculiar tropism for specific organs, occupying the entire coelomic cavity, as confirmed in the two cases found in July. Moreover, the life-cycle of *Philometra* spp. is characterized by ovoviviparity and the production of larvae being directly spread in the water column from the nematode reproductive system or by adult female destruction due to the rapid hypotonic effect of the surrounding water (Moravec, 1977, 2006), as highlighted in the two saddled seabream found in August. Therefore, adults of *Ph. obladae* are localized within the coelomic cavity, joining the water column through the cloaca, producing severe injuries to coelomic organs, thus compromising physiological functions, which are partially superimposable with parasitic castration due to gonadal pressure due to other philometrids (Molnár, 1967; Moravec, 1977).

Considering the data reported by Burak Daban et al. (2020) on the development of *O. melanura*, the specimens investigated in the present study were estimated as *O. melanura* juveniles (< 1 year of age). Moreover, considering the homogeneity of the fish stock present in the examined area, we can confirm that juveniles of *O. melanura* are more sensitive to infection with *Ph. obladae* compared to older specimens. This parasite was morphologically described for the first time (Moravec et al., 2008a) in *O. melanura* caught off Sicily, Tyrrhenian Sea, Central Mediterranean, exactly in the area of the present study. The size of the infected type-host specimen was 18 cm, considered according to specialized studies (Pallaoro et al., 2003; Mahmoud, 2010) as a subadult fish. Thus, this record improves the current knowledge on the specificity of *Ph. obladae* confirming that *O. melanura* is susceptible to this infection. As reported by Pallaoro et al. (2003), the diet of juvenile *O. melanura* is composed mainly of crustaceans, considered to be potential intermediate hosts of *Ph. obladae*, highlighting the importance of further studies on the life-cycle of this parasite, to better describe the host-parasite interaction.

Regarding the morphological characteristics of the gravid females of the present study, the specimens key down to *Ph. obladae* in the key of Moravec et al. (2008a) based on the presence of a well-developed anterior oesophageal inflation, the lack of caudal projections, and a body length greater than 130 mm. Morphological features of the present specimens also agree with the original description of *Ph. obladae* but show some morphometric differences. Thus, the present gravid female specimens are smaller (170–202 vs 335 mm) but all have both larvae and eggs in the uterus. The oesophagus has the characteristic anterior bulbous inflation but is longer in comparison with the gravid specimen of *Ph. obladae* described by Moravec et al. (2008a) (1.5–2.1 vs 1.29 mm,

respectively). The larvae studied by us showed the same length (550–600 vs 549–600 µm) reported by Moravec et al. (2008a). Considering that *Ph. obladae* was described based on a single gravid female collected from the locality of origin of the present specimens, we believe these differences represent intraspecific variation. Our data thus expand the ranges of morphometric variation of *Ph. obladae*.

The present report also confirms the absence of male specimens in the studied area during the summer, highlighting the necessity to focus attention also on late spring, in accordance with the data reported by Gaglio et al. (2009).

5. Conclusion

The present study provides new data about the swimming impairment caused by *Ph. obladae* in wild fish. The atypical swimming behaviour of infected *O. melanura* observed during diving activities, considering the presence of an operator, confirms the significant impact on fish health status. Future studies are necessary to better understand the dynamics of *Ph. obladae* infection in *O. melanura*, as well as its epidemiology, molecular evaluation and male specimen description.

Ethical approval

No procedures on live animals were carried out; thus, the requirement for ethical approval was not applicable.

CRediT authorship contribution statement

Giovanni De Benedetto: Conceptualization, Methodology, Formal analysis, Data curation, Resources, Investigation, Writing - original draft, Writing - review & editing. **Gabriella Gaglio:** Conceptualization, Methodology, Formal analysis, Data curation, Resources, Investigation, Writing - original draft, Validation.

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Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data supporting the conclusions of this article are included within the article and its video files.

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