Fisheries Research 209 (2019) 178–185

Contents lists available at ScienceDirect

Fisheries Research

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

Morphological and genetic identification of *Pennella instructa* (Copepoda: Pennellidae) on Atlantic swordfish (*Xiphias gladius,* L. 1758)

María Llarena-Reino[a,b,*], Elvira Abollo[c], Santiago Pascual[a]

[a] Ecobiomar, Instituto de Investigaciones Marinas de Vigo, CSIC, C/Eduardo Cabello 6, 36208 Vigo, Spain
[b] CESAM & Dep. Biologia, Universidade de Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal
[c] Centro Tecnológico Del Mar (Fundación CETMAR), C/Eduardo Cabello s/n, 36208 Vigo, Spain

ARTICLE INFO

Handled by J. Viñas

Keywords:
Swordfish
Xiphias gladius
Pennella instructa
Morphology
Genetic

ABSTRACT

During the last years the presence of parasites in commercial fish species has increased becoming an emergent major sanitary problem. For seafood companies the control of this biological hazard is turning into a priority issue, as quality of the products is now being seriously compromised. The swordfish *Xiphias gladius,* as one of the most important commercial species among European markets, has been inspected for the presence of pennellids. A total of 214 mesoparasitic copepods were sampled for epidemiology and genetic analysis, 5 pennellid cephalothoraxes' for morphological and genetic studies and 50 slices of parasitized swordfishes were examined for internal lesions and diagnostics on the basis of morphological characters. All hosts were captured in Atlantic waters. *Pennella instructa* was morphologically identified as the only metazoan species in the 5 cephalothoraxes. Morphological characters and internal and external lesions caused by *P. instructa* were acutely described. Prevalence and mean Intensity of infection were determined for the total fishes parasitized. Copepods were molecularly characterized and the phylogenetic relationships were explored based on 18S and 28S rDNA sequences. Phylogenetic analyses using maximum likelihood models were performed including pennellid external portions and cephalothoraxes (morphologically identified as *P. instructa*). All pennellid samples were clustered into the same clade with bootstrap values of 100% for both genes sequenced. The prevention of rejections due to these parasites must be the first step to ensure safer and high quality standard products to final consumers. Monitoring actions and proactive self-inspections including preventive and corrective measures should be more intensively integrated into HACCP systems of seafood companies.

1. Introduction

The swordfish *Xiphias gladius,* is one of the most important species among European commercial fish stocks. This pelagic species which extends from tropical to temperate-cold areas (Nakamura, 1985; Mattiucci et al., 2005; Garcia et al., 2010) is usually caught in Macaronesia, involving the whole area between Cape Verde and Azores. The waters close to Portugal and Spain are probably the most frequented fishing areas during the autumn months. On the contrary, March and April are the months when the fishing vessels increase their captures far from those coasts.

Despite the considerable economical importance that characterises the fishing ports of Portugal as Peniche, Âncora, Povoa de Varzim, Sesimbra or Algarve, many Portuguese fishing vessels dedicating their activity to swordfish often unload the fish catches in the fishing port of Vigo (Spain). Among other reasons, this is due to the fact that the swordfish is the 10th species of fresh fish most unloaded in that port, as the Fishing Statistical Information of the 2012 Annual Report from the Port Authority of Vigo states (Port Authority of Vigo, 2012). Vigo's port is the most important fishing harbour of Spain and one of the most relevant ports in Europe in terms of catches. In addition, the commercial interest that *X. gladius* has on Spanish fishing sector and among consumers is greater than the Portuguese existing one, possibly due to cultural and gastronomic reasons.

Parasites are an important component of global biodiversity and copepods in particular are highly abundant and prevalent mainly as fish parasites (Morales-Serna et al., 2012). The members of the family Pennellidae, order Siphonostomatoida, are mesoparasites characterised by needing intermediate hosts in their life cycle (Kabata, 1979; Abaunza et al., 2001). Closely related to swordfishes, the genus *Pennella* probably constitutes the most significant threat for this commercial fish species since it is becoming an emergent sanitary problem. However,

* Corresponding author at: Ecobiomar, Instituto de Investigaciones Marinas de Vigo, CSIC, C/Eduardo Cabello 6, 36208 Vigo, Spain.
E-mail address: mllreino@yahoo.es (M. Llarena-Reino).

https://doi.org/10.1016/j.fishres.2018.09.012
Received 3 April 2018; Received in revised form 11 August 2018; Accepted 12 September 2018

the difficulty in obtaining individuals for description and their phenotypic plasticity (Abaunza et al., 2001), make this parasite species one of the least known in its family. Some of the morphological characters used to differentiate *Pennella* species are the type of host parasitized, overall length of the parasite, shape, size and configuration of cephalothorax papillae, segmentation of the first and second antennae, lateral horn number, shape and configuration, and structure of the abdominal plumes (Hogans, 2017). The great concern that this parasite is causing among fishing companies is strengthening the increasing control of this biological hazard.

The aim of this work was to determine the infection levels of the pennellid specimens that are infecting the swordfish population in five main fishing areas of Eastern Atlantic waters, their distribution by anatomical regions in the host body surface, and their morphological and genetic identification. This attempt to increase our knowledge about this problem intends helping to develop effective solutions in the very short term and gives the fishing sector the chance to plan proactive measures and put them into operation, in order to minimize the increasing damage of quality of fishery products and the high economic losses that consumer rejections causes.

2. Materials and methods

2.1. Host inspection and parasite collection

A total number of 1631 swordfishes, *X. gladius,* caught in the NE Atlantic fishing areas FAO 27 and FAO 34, comprising the whole Macaronesia, from Cape Verde to Azores and the waters close to Portugal and Spain, were externally examined for the presence of pennellid copepods. Five sampling grounds were defined in order to have a more organized representation of the parasites collected. Taking into account the specific geographical origin of the hosts, 31 pennellids from “A” area, 35 from “B”, 33 from “C”, 32 from “D”, and 41 from “E” area where classified for further study (Fig. 1). In some cases it was not possible to obtain specific fishing subarea of hosts.

Hosts inspections took place in the fish auction market of the fishing port of Vigo (Spain) during 17 sampling days coinciding with the days of higher tonnage of landed fish, between March and September of 2011. Throughout that 6-months period, a total of 15 Portuguese and 5 Spanish long line vessels were the main swordfish providers at port. Hosts’ body surface, natural orifices, gills, and fins were externally inspected for pennellids. All the external parasitic portions found (including trunk, abdomen with brush and egg strings), were measured and collected without any kind of manipulation of hosts’ bodies due to fish auction market’s regulations. Parasite specimens were preserved in 96% ethanol until genetic studies. All data concerning capture information or biological characterization of parasitized hosts was registered and is summarized in Table S1 (Supplementary material). Lower jaw furcal length (LJFL), fork length (FL) and the round weight (RWT) were measured and recorded respectively from all parasitized swordfishes. In addition, information such as sampling date, port of origin of vessels, hosts fishing area and subarea and length of parasitic external portions, is also provided in the table.

As the Commission Regulation (EC) No. 2074/2005Commission Regulation, 2005Commission Regulation (EC) No. 2074/2005 states in Section 1 of Annex II, a representative number of fishes has to be routinely submitted to a visual inspection by qualified technicians, at establishments on land and on board factory vessels. Persons responsible of that kind of measures must determine the scale and frequency of inspections depending on the type of the fish products, their geographical origin, and the final use they are intended for. Accordingly, fifty parasitized slices provided by the quality control department of a Spanish fish processing, distribution and trade company, were examined for the presence of pennellid copepods. Slices belonged to six commercial swordfishes caught in May of 2012 in fishing grounds from NE Atlantic fishing areas FAO 27 and 34. Slices were photographed and cysts were carefully measured in maximum wide and length. Pennellid cephalothoraxes contained inside cysts of slices were frozen at a temperature of −20 °C until dissection procedures, morphological description and identification. Subsequently, they were preserved in 96% ethanol for posterior genetic studies. Cephalothoraxes sampling was carried out between May and July of 2012, and restricted detailed

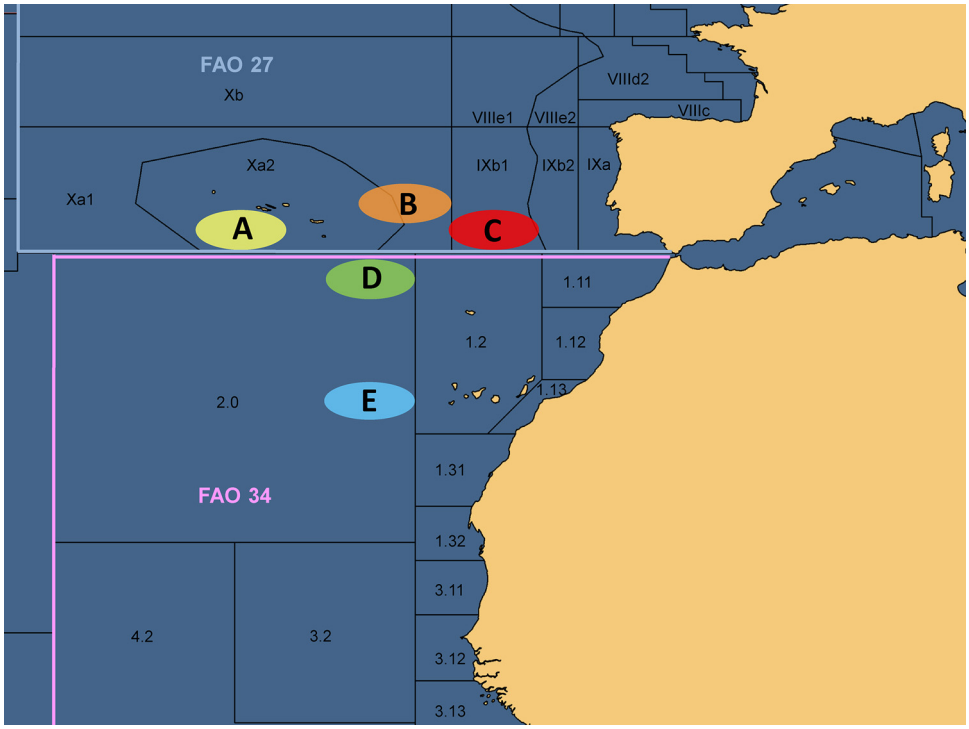

Fig. 1. Map of partial NE Atlantic fishing areas FAO 27 and 34 with delimited subareas including the geographical origin of parasitized hosts. Pennellids analyzed for genetics have been grouped by their location in the five specific sampling grounds highlighted (A, B, C, D and E).