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Impact of *Anisakis pegreffi* Infection on Gonadal Health and Gonadosomatic Index of European Hake (*Merluccius merluccius*)

Alaa Eldin Eissa[1], Reda M.S. Korany[2], Rabia A. El Zlitne[3], Ayad A. Magdy[4], Mahmoud S. Sharaf[1], Awad A. Abdelbaky[1], Abeer E. Mahmoud[5], Rehab R. Abd ElMaged[6], Asmaa M. K. Edrees, Rehab A. Qorany[5], Doaa M. Faisal[5], Ebtehal E. Hussein[8], Hatem H. Mahmoud[9], Hisham M. Elgazzar[10], Said El Behiri[11], Emad A. Afify[12], Abdelbary Prince[13], Marwa M. Attia[14]

[1]Department of Aquatic Animal Medicine & Management, Faculty of Veterinary Medicine, Cairo University, Egypt
[2]Department of Pathology; Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
[3]Department of Preventive Medicine, Faculty of Veterinary Medicine, University of Tripoli, Libya
[4]Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Libya
[5]Department of Fish Diseases, Animal Health Research Institute, Agriculture Research Center, Dokki, Egypt
[6]Department of Parasitology, Animal Health Research Institute, Agriculture Research Center, Dokki, Egypt
[7] Department of Fish Diseases & Management, Faculty of Veterinary Medicine, Beni-Suef University, Egypt
[8] Poultry and Fish Nutrition Department, Faculty of Agriculture, Menufia University, Egypt
[9] Arab Academy for Science, Technology and Maritime Transport, Alexandria, Egypt
[10]Lake and Fish Resources Protective and Development Agency, 5th Settlement, Cairo, Egypt
[11]Dakahlia Poultry Co, El Nasr Road, Nasr City, Cairo, Egypt
[12] DSM Nutritional Products Ltd, Wurmisweg 576, 4303 Kaiseraugst, Switzerland
[13]Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Cairo University, Egypt
[14]Department of Parasitology; Faculty of Veterinary Medicine, Cairo University, 12211, Giza, Egypt
*__Corresponding Author:__ Alaa Eldin Eissa, **E-Mail:** aeissa2005@gmail.com; aeissa2012@cu.edu.eg

ABSTRACT

Parameters belonging to the physical status and gonadal size of certain fish provide crucial information for assessing both the productivity and fecundity of declining fish populations. These parameters are vulnerable to the negative impacts of disease agents such as internal or systemic parasites. Although parasitic diseases might influence these parameters, the literature investigating these pathophysiological alterations is scanty. Therefore, the current study represents one of the scarcest studies that document the possible link between parasitism, gonadal health, and the growth of European hake (*Merluccius merluccius*). Screening of imported European halves indicated a relatively high prevalence of *Anisakis pegreffi* Larvae 3 infestation, with an 80% prevalence rate, a mean intensity of 24.4, and a mean abundance of 19.5. However, the prevalence of Anisakid larvae infection in native fish was 36% with a mean intensity of 7.36 and mean abundance of 2.65.The current research revealed remarkable ovarian pathology that involved several forms of degenerative changes in ovarian tissues. Such gonadal pathologies were attributed to the damaging effect of the retrieved *Anisakis pegreffi* Larvae 3. Gonadosomatic index of both heavily infected imported / native hakes was relatively impacted by the progressive gonadal pathology resulting from *Anisakis pegreffi* L3 infection. Morphometric measurements of the gonads and body have revealed that, gonadosomatic index of both heavily infected imported / native hakes was relatively impacted by the progressive gonadal pathology resulting from *Anisakis pegreffi* L3 infection.

Keywords: *Anisakis pegreffi*, Gonadosomatic index, Gonadal pathology, *Merluccius merluccius*.

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INTRODUCTION

The Mediterranean Sea, the southern shore of the Black Sea, and the Atlantic coasts of Europe and western North Africa make up the large geographic distribution of the European hake (*Merluccius merluccius* (**Linnaeus, 1758**). Hake is one of the most significant target species for commercial fishing in the Western Mediterranean (**Oliver and Massut, 1995**). All Mediterranean stocks are currently regarded as being severely exploited, and in some regions, such as the Gulf of Lion, hake stocks may have declined past safe biological limits due to a the increased exploitation through fishing.

Both the Mediterranean and the Atlantic stocks of hake have been shown to harbour metazoan parasites. On the one hand, helminth parasites (monogeneans, digeneans, cestodes, nematodes, and acanthocephalans) identified in hake have been the subject of more than fifty references compiled by **Dolors Ferrer-Maza *et al.*, (2014)** for the British natural history museum's host-parasite database Since then, genetic studies have dominated research on helminths, mostly nematodes, in hake (**Mattiucci *et al.*, 2004; Farjallah *et al.*, 2008; Ceballos-Mendiola *et al.*, 2010**). On the other hand, hake parasite copepods have also been observed regularly (**Grabda and Soliman, 1975; Raibaut *et al.*, 1998; Gaglio *et al.*, 2011**). Nevertheless, till date there are few studies available focusing on large range of parasites present in hake and their possibly harmful effects.

In Egypt, European hake is one of the most consumed fish for its gonads, which are a common source of infection. Despite the wide consumption rates of hake by many people in coastal areas of Egypt, there is no many records of human anisakiosis (**Muñoz-Caro *et al.*, 2022**.

The Anisakidae family of nematode parasites (Nematoda) is thought to have some of the most significant biological effect in seafood products (**Ishikura *et al.*, 1993**). Human anisakiasis is a seafood-borne parasitic disease that resulted from unintentional ingestion of live *Anisakis* larvae that infect the edible sections of fish or squid (**Audicana and Kennedy, 2008**). Consumers, food safety authorities, and the fishing industries are all gravely concerned about the potential risks to human health posed by the presence of these parasites in fish (**Mattiucci *et al.*, 2014**).

The two genera most frequently linked to anisakiasis are Anisakis and Pseudoterranova, despite the fact that cases of human infection have been documented with worms from a variety of species within these families (**Bouree *et al.*, 1995**). Fish and squid function as intermediate and/or paratenic hosts, whereas crustaceans operate as first intermediate hosts, transmitting the larvae to the corresponding cetacean final hosts (**Mattiucci and Nascetti, 2006; Mattiucci *et al.*, 2018;**). Some Anisakis species may migrate into the muscle, suggesting a possible zoonotic risk (**Mattiucci *et al.*, 2018**). The majority of Anisakis larvae dwell encased in the viscera of fish.

Anisakis larvae have been divided into 4 clades and can be distinguished molecularly (**Mattiucci and Nascetti, 2008**). These clades are clade 1, that includes *Anisakis simplex sensu stricto*;*A. pegreffii* and*A. berlandi*; while the clade 2 includes*A. ziphidarum* and *A. nascetti*. Clade 3 includes *A. physeteris*; *A. brevispiculata* and *A. paggiae*. Clade 4 includes one member which is*A. typica*.

Anisakis simplex, *A. pegreffii*, and *A. typica*, in particular, have been isolated from a several range of fish around the world, including mackerel (**Eissa *et al.*, 2018**), European anchovy (**Mladineo *et al.*, 2012**), European hake (Cipriani et al. 2015), and Atlantic herring (**Costa *et al.*, 2016**).*A. pegreffii* appear to be the only species capable of moving intra-vitam and post-mortem into the fish flesh out of the nine known *Anisakis* species (**Mattiucci and Nascetti, 2008; Mattiucci *et al.*, 2009; Mattiucci *et al.*, 2018**). Additionally, according to genetic studies they are the only species that can cause human anisakiasis.

Anisakiasis has been reported more frequently in Korea, Australia, China, Croatia, and the United States of America due to the internationalization of regional food, the improvement of diagnostic methods, and increased awareness. Only a few occurrences of human anisakiasis in Norway have been linked to consumption of raw or minimally cooked fresh seafood. A German-American woman from the United States of America had the first anisakid discovered in her endo-cervical adenocarcinoma in 2014, while a toddler in Croatia experienced anisakiasis for the first time in 2014 that was initially misdiagnosed as a bowel obstruction (**Aibinu *et al.*, 2019)**. The aims of this study were to assess the prevalence of *Anisakis* spp and evaluate the gonadal health as well as gonadosomatic index of wild caught European hake.

MATERIALS AND METHODS

Fish Sampling

From January to December 2022, random monthly samples of *Merluccius merluccius* were taken from the landings at the fishing ports of Damietta, Rashed, and Alexandria as well as from commercial deep-water trawl fishing vessels. 1187 European hake, *Merluccius merluccius*, were investigated in total. The total weight and length of the fish gathered for the current investigation ranged from 33.34 to 2,940 g and 17 to 70 cm, respectively. A subsample was taken to the laboratory to be dissected as well as to measure the gutted and gonad weights in grams. Moreover, fish sex and the sexual maturity stage were examined. Otoliths were removed and stored in vials for subsequent examination relevant to age determination. Each examined fish was given a serial number that was recorded in a logbook containing all the information related to that fish.

Parasitological examination

Through visual inspection of gonads, nematode larvae were found encapsulated or free within the gonadal tissues. Larvae were rinsed in phosphate buffered saline (pH 7.2) solution, and then maintained at 4 $^{\circ}$C. The obtained nematodes were cleaned with lactophenol, mounted with glycerin jelly, and examined using a stereomicroscope connected to a computer with an optical digital USB camera (**Eissa *et al.*, 2020**). The morphological characteristics and measurements of the lips surrounding the anterior end, the presence or absence of a boring tooth, the shape of the esophageal ventriculus, the shape of the post-anal tail and its mucron or spine were used in the identification of the anisakid nematodes. These criteria were adopted from Anisakis morphological criteria published by**Eissa *et al.*, (2018) and Abdelsalam *et al.*, (2020)**.

Prevalence, mean intensity and abundance and statistical estimation

$$\text{Prevalence (\%)} = \frac{\text{No. of Infected fish}}{\text{Total number of fish examined}} \times 100$$

$$\text{Mean Intensity} = \frac{\text{No. of collected parasite(s)}}{\text{No. of fish infected}}$$

$$\text{Abundance} = \frac{\text{Number of parasites(s)}}{\text{Number of fish examined}}$$

The 95% confidence interval (CI) was calculated in respect to the prevalence.

Histopathological examination

Gonadal tissues were collected, fixed in 10% neutral buffered formalin (Solmedia, UK), washed, dehydrated, cleared and embedded in paraffin. The paraffin embedded blocks were sectioned at 5 micron thickness and stained with Hematoxylin and Eosin (**Bancroft and Gamble, 2008**).

RESULTS

Taxonomic summary

Parasite:

Anisakis Pegreffi larvae type1 **(Rudolphi, 1809 det. Krabbe, 1878)**

Family

Anisakidae (**Railliet and Henry, 1912)**

Site of infection: Third stage larvae were found in both gonadal tissues and encapsulated on the serosal surface of ovaries and testes of infected fish.

Prevalence of infection, intensity means, and mean abundance for imported fish

Forty out of fifty imported fishes were positive for Anisakid larvae, giving 80% prevalence rate with a mean intensity of 24.4 and mean abundance of 19.5. However the prevalence of Anisakid larvae infection in native fish was 36% with a mean intensity of 7.36 and mean abundance of 2.65.

Morphological characters

Anisakis pegreffi larvae were identified from both native and imported *M. merluccius* (Nematoda: Anisakidae; Dujardin, 1845). Larvae had cylindrical / elongated shape with two attenuated ends. At their widest point, they measured 0.54 - 0.8 mm long and 18.5- 23.6 mm long. Three unnoticeable lips and a noticeable boring tooth encircled the oral apertures on the anterior end (Fig. 1A-B). The ventriculus glandular portion of the oesophagus measured 0.95-1.55 (1.25±0.5) mm in length, whereas the anterior muscular portion was 1.75-2.9 (1.99± 0.67) mm long. The oblique esophago-gastro-intestinal junction (ventriculus) measured 0.80–1.0 (0.95±0.502) (Fig.1C). A transversely striated cuticle covered the whole larva (Fig. 1D).

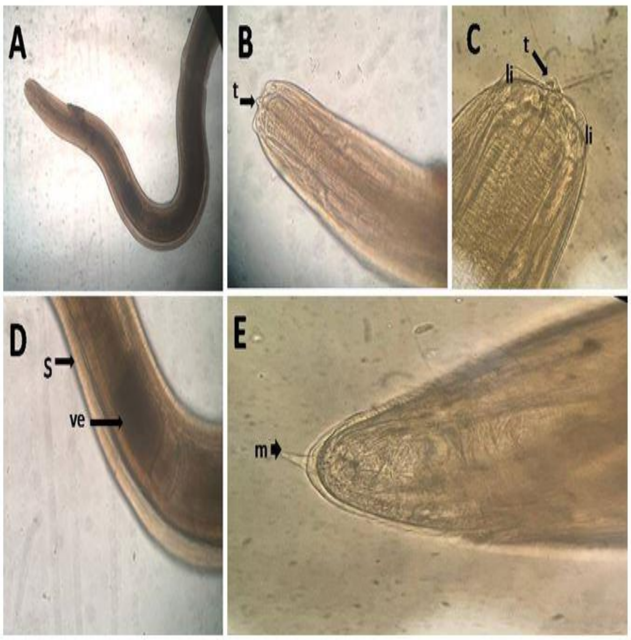

Fig.1: *Anisakis pegreffi* 3rd stage larvae. A: anterior end showing; three inconspicuous lips with prominent boring tooth (t); B;C: light microscopic photo of anterior end showing prominent boring tooth (t) and lips (li) C: light microscopic photo of an esophago-intestinal junction (Ventriculus (ve)) was oblique referred by arrow with transverse striated cuticle (s). E: light microscopic photo of larvae showing a short mucron (m) with triangular posterior end.

Gonadosomatic index (GSI) was calculated according to **Sokal** *et al.*, (**1969**) using the following formula:

GSI = (GoWe /W) *100

Where: GoWe: the gonad weight in (g). W: is the gutted weight in (g).

For both males and females, monthly fluctuations in the value of GSI were measured in order to pinpoint the spawning season. For both sexes, the gonadosomatic index (GSI) was calculated every month from January to December of 2017. It is clear that throughout the year, females have higher average GSI levels than males. It was noted that the average monthly values of GSI began to rise gradually in December with a value of 1.1%, elevated to 1.7 in January, and reached their greatest value in February (2.9%). Then, following a sudden decline in October, it fell to (0.33%), where infection frequency was still rather high.

The GSI in males started to increase progressively in January till it reaches a high value of 1.18 in February. It decreased in November, after which another progressive increase took place in the value of male GSI to reach a maximum value in June, followed by sharp drop in August where prevalence of infection was still high. Summary of GSI in both males and females *M. merluccius* from is shown in Table 1 and Fig.2.

Table 1: Monthly variation of the gonadosomatic index (GSI) (mean ±SD) for *M. merluccius* from the Egyptian Mediterranean water (2017).

Month	GSI %	
	Females	Males
January	1.67±2.77	0.96±0.41
February	2.93±3.43	1.18±0.20
March	1.77±1.84	0.62±0.04
April	1.73±2.91	0.58±0.16
May	0.97±0.33	1.07±0.42
June	0.86±0.31	1.08±0.76
July	0.76±0.19	0.76±0.18
August	0.64±0.14	0.43±0.09
September	0.67±0.42	0.48±0.10
October	0.33±0.02	0.50±0.26
November	0.52±0.03	0.30±0.03
December	1.12±1.41	0.42±0.16

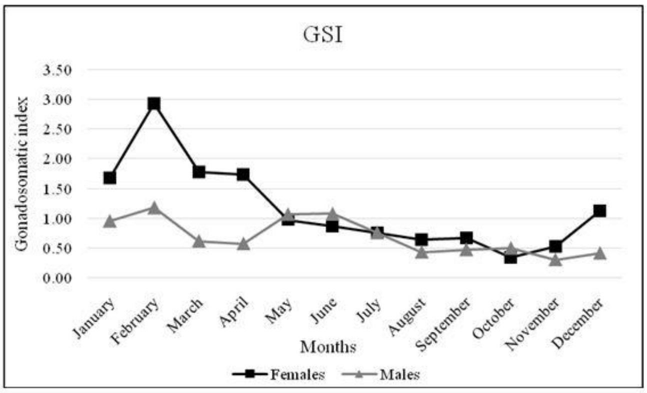

Fig.2: Monthly variation of the gonadosomatic index (GSI) for infected *M. merluccius* with *A. pegreffi*

Histopathological findings

Histopathological examination of infested ovaries revealed presence of Anisakis larvae in ovarian tissue (**Figs. 3 a, b**), also infested ovarian tissue showed various tissue reaction as fibrosis (**Fig. 3c**), degeneration of some oocytes (**Fig. 3d**), thickening and congestion of interstitial blood vessels (**Fig. 3e**), interstitial edema (**Fig. 3f**) with infiltration of interstitial tissue with mononuclear inflammatory cells (**Fig. 3g)**.

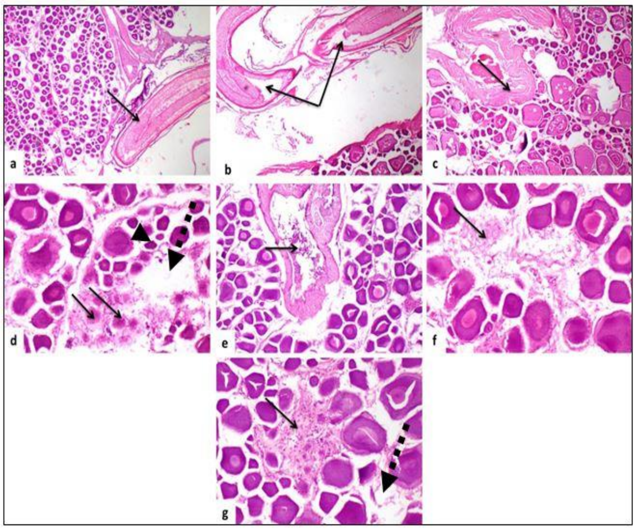

Fig. 3: Photomicrograph of ovaries showing, (a) presence of mature anisakis nematode in ovarian tissue (arrow) (H&EX100), (b) presence of multiple nematodal worms (arrows) (H&EX200), (c) fibrosis of interstitial tissue (arrow) (H&EX200), (d) degeneration and necrosis of some oocytes (arrows) (H&EX400), (e) thickening and congestion of interstitial blood vessels (arrow) (H&EX200), (f) Interstitial edema dispersing the interstitial tissue(arrow) (H&EX400) and (g) edema of interstitial tissue with infiltration of mononuclear inflammatory cells (arrow) (H&EX400).

DISCUSSION

Endoparasites have been described as one of the significant pathogens affecting reproductivity of fish. The current study highlights the impact of anisakid nematodes on hake condition and reproduction (**Dolors Ferrer-Maza *et al.,*** 2014). There may also be a synergistic effect throughout the developing period, as evidenced by the negative correlations between gonad energy reserves and both the overall number of species and the total number of endoparasite species **(Dolors Ferrer-Maza *et al.,* 2016)**.

Anisakid nematodes in the mesenteries of hake appeared to adversely affect the energy reserves in the liver and, in particular, the nematode *A. pegreffii*, which affects the energy reserves in the gonads. Additionally, a high intensity of infection by *A. pegreffii* may affect the gonadal energy reserves when hake reaches the spawning-capable phase (**Dolors Ferrer-Maza *et al*., 2014)**. The individual intensity of infection plays an important role in terms of the adverse effects of parasitism. Furthermore, during the spawning-capable phase, the presence of anisakid nematodes in the mesenteries was related to high values of the intensity of atresia. Such a relationship may be due to a trade-off aimed at partially compensating for some of the energy taken by the parasites **(Dolors Ferrer-Maza *et al.,* 2016).**

In Egypt, the prevalence of *Anisakis* spp. larvae varied depending on the fish host, reaching 36.6% in European hake (**Abou-Rahma *et al.,* 2016**), 92.3% in mackerel (**Abdel-ghany 2011**), 23-72.8% in Mediterranean sand smelt (**Samir *et al.,* 2015**) and 70% in Orange Spotted Trevally (**Arafa *et al.,* 2019**).

The Atlantic Ocean, as well as the East and West Pacific Oceans, are home to the majority of the *Anisakis* spp. in Clade I. Species distribution of *A. pegreffii* and *A. simplex* are widespread in the Mediterranean where both species have been detected in the sea, in addition to the Arctic Circle where they both live and down to the oceans off Antarctica. Various anisakid from a single parasitized fish species have been reported. For instance, Chub mackerel, or *Scomber japonicus*, was found to be susceptible to larvae of *A. simplex* and *A. pegreffii*.

Japan, Europe, and nations in Africa and South America are among the geographical areas where human anisakiasis is prevalent. In these areas, anisakids are frequently discovered in locally caught fish. For instance, in northern Morocco, *Trachurus trachurus* (horse mackerel) captured in both Atlantic and Mediterranean waters have been discovered to contain *A. pegreffii*, but no *A. simplex* has ever been isolated from this species of fish. *Anisakis spp* have been discovered in many different fish species from the Ligurian Sea in Italy, but *A. pegreffii* was discovered with a significant prevalence in *T. trachurus*. *A. pegreffii* is also present in *A. simplex* and *Todaropsis eblanae* (the smaller flying squid), *Todarodes sagittatus* (the European flying squid), and *Todarodes angolensis* (the Angolan flying squid) (s.s.); **Eissa *et al.,* 2021.**

A few investigations have been made by researchers on the reproductive biology of *Merluccius merluccius* in the Mediterranean waters. The reproductive traits of *Merluccius merluccius* in the Central Mediterranean Sea area were studied by many authors in the central region **(Bouhlal, 1973; Perez-Villarreal *et al.,* 1987; Tornaritis *et al.,* 1993; Soriguer *et al.,* 1997; Recasens *et al.,* 1998; Khoufi *et al.,* 2014; Carbonara, Porcu, *et al.,* 2019)**, and in the eastern Mediterranean Sea area **(Mugahid *et al.,* 1982; Al-Absawy, 2010; Kahraman *et al.,* 2017b)**, while in the western Mediterranean Sea area **(Bouaziz *et al.,* 1998; Belhoucine *et al.,* 2012) .**

Studies on *Merluccius merluccius* sex ratio reported that there is a high differences in the sex ratio within the Mediterranean Sea, north and south Atlantic, these differences may be attributed to local factors like habitat variation, salinity, water temperature, food availability, maturity stage, fishing season and value of fishing mortality **(Ricker, 1968; BagenalandTesch, 1978; Recasens *et al.,* 1998; Basilone *et al.,* 2006; Froese, 2006)**. Whilst, the sex ratio in the Mediterranean Sea has stayed constant over recent years **(Aldebert *et al.,* 1996)**.

Notably, it has been proposed that *A. simplex* is primarily responsible for anisakiasis in Japan since it appears to penetrate muscle tissue more quickly than *A. pegreffii*, resulting in a higher exposure rate when mackerel is consumed. While more species were separated from fish in northern than in southern locations, the diversity of various anisakid species in parasitized fish declined with latitude; Eissa et al. 2021.

The Egyptian Mediterranean fisheries landings consists of many target species from pelagic fishes like sardines, anchovies, horse mackerel, jacks, blue fishes, mullet, chub mackerel, Atlantic mackerel and tuna **(Mehanna *et al.,* 2005)**.The landings of *M. merluccius* in the Egyptian Mediterranean had not been officially recorded among the commercial fish species landings until 2016. The first time it was included in GAFRD's statistical year book in 2017 and the total landing of *M. merluccius* was 270 tonnes, then 677and459tonnes in 2018and 2019, respectively **(GAFRD, 2019)**.

M. merluccius has a rather long spawning period which extending from December to early June. For the length at first sexual maturity it was 31cm with a percentage of 53.3%. The monthly fluctuations of the GSI values increased gradually from November to January **(Al-Absawy, 2010)**.

Studies on the reproductive biology of *M. merluccius* in the Egyptian Mediterranean waters showed that the males dominated the catch in the younger length groups from 13 cm to 32 cm while the females dominated catch for the larger length groups over 32 cm with a total sex ratio (1: 0.596) (M:F). For the length at first sexual maturityit was 24 cm for females an d 21 cm for males (**Philips and Ragheb, 2013)**.

A single reproductive event that lasted the entire year, from November to March with a peak in February, appears to be defined by the GSI monthly trend. The spawning season, as estimated for the Egyptian Mediterranean Sea, covered the months of November and March, as reported in the western and central Mediterranean Seas **(Carbonara, Porcu, *et al.*, 2019),** the eastern and central Atlantic coasts of Morocco **(Habouz *et al.*, 2011),** and the Sea of Marmara in Turkey **(Kahraman *et al.*, 2017).** The peak of GSI occurred in June, according to research by other authors who studied a different spawning season in the Adriatic Sea **(Michela *et al.*, 2017).**

These parasites have historically been far more common in developing nations than in wealthy ones. Through their anti-inflammatory or immuno-modulatory excretory/secretory (ES) molecules, some nematodes, such as *Necator americanus* (hookworm) and *Trichuris trichiura* (whipworm), are actually thought to be helpful in preventing the development of allergy and other chronic inflammatory conditions, such as colitis.

CONCLUSION

Overall, our findings appear to support the theory that hosts and parasites are in a state of dynamic equilibrium (**Barret, 1986**), as strong metazoan parasite effects on hake health and reproduction were not found within the observed infection intensities. Nevertheless, no brand-new parasites that could cause hake to develop a novel immune response and eventually lose their physical health or reproductive ability have been discovered to date. In this sense, and taking into account new species interactions resulting from the biological effects of global climate change and anthropogenic change, we consider it important to continue monitoring parasitism in commercially important species like hake, as well as the potential additive or synergistic effects on hake condition and reproduction resulting from a future increase in the parasite load.

Ethics approval: Is not applicable as we have dealt with caught wild fish (European hake) throughout the study.

Competing interest: There is no conflict of interests of any sort between authors or elsewhere.

Author Contribution: All authors equally contributed to the current manuscript

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