Anisakid nematodes of the blackspot seabream, Pagellus bogaraveo, from Madeiran waters, Portugal

Article *in* Acta Parasitologica · June 2004

CITATIONS
31

READS
539

4 authors, including:

Graça Costa
Universidade da Madeira
45 PUBLICATIONS **828** CITATIONS
SEE PROFILE

Stefano D'Amelio
Sapienza University of Rome
214 PUBLICATIONS **4,694** CITATIONS
SEE PROFILE

Acta Parasitologica, 2004, 49(2), 156–161; ISSN 1230-2821

Anisakid nematodes of the blackspot seabream, *Pagellus bogaraveo*, from Madeiran waters, Portugal

Graça Costa[1*], Ana Madeira[1,2], Tânia Pontes[1,2] and Stefano D'Amélio[3]

[1]CEM, University of Madeira, Campus da Penteada, 9000-390 Funchal; [2]Regional Environmental Department, Funchal, Portugal; [3]Department of Science and Public Health, Section of Parasitology, University of Rome "La Sapienza", Piazzale Aldo Moro, 5, 00185 Rome, Italy

Abstract

Two hundred and eighty-one anisakid larvae were found encapsulated in stomachs and mesenteries of 69 blackspot seabreams, *Pagellus bogaraveo* from Madeiran waters. Ninety-four larvae were identified as *Anisakis simplex* s.s., *Anisakis pegreffii* (Type I larvae), *Anisakis simplex* s.l. (Type II larvae) and *Hysterothylacium* sp. Prevalence of infection with anisakids increased with host length from 81.3% to a maximum of 96.3%. Mean intensity ranged from 3.5 (at length class 25 cm) to 4.8 (at length class 35 cm), with the majority of fish infected with only 1 or 2 parasites. A positive but not significant correlation was found between intensity and length ($r_s = 0.0419$, $p = 0.717$). The high values of prevalence and low values of intensity may indicate that the anisakid larvae are dispersed within their hosts. No particular histopathological lesions were found associated with the presence of the nematodes, corroborated by a positive but not significant correlation that was found between intensity and condition factor ($r_s = 0.242$, $p = 0.035$).

Key words

Anisakid nematodes, fish, *Pagellus bogaraveo*, Sparidae, Madeira Island

Introduction

The blackspot seabream, *Pagellus bogaraveo* (Pisces, Teleostei, Sparidae), is a fish species with high commercial value, in Madeira (7.5 Euros/kg compared with 2.0 Euros/kg for mackerel, *Scomber japonicus* and black-scabbard fish, *Aphanopus carbo*, data taken from the landing registry of the Regional Fisheries Board of Madeira for 2001). It has a geographical range extending through the Mediterranean and Atlantic Ocean from Norway (65°N) to Cape Blanco, Madeira and the Canaries (30°N) (Bauchot and Hureau 1986). In Madeira, landings of this species, showed some slight fluctuations from 2000 to 2001 (0.34 and 0.30% of total fish landings, data from the Regional Fisheries Board of Madeira). Fluctuations in fish populations may be related to an increase of the fishing pressure (Iversen 1996) or to the occurrence of parasites and diseases (Lom and Dyková 1992, Molnár *et al*. 1993, Scholz 1999). Several parasites are known to inflict some sort of impact to their fish hosts in their natural habitat, such as increasing their susceptibility to predation, decreasing host density (Mo 1994) reducing the marketability of fish (Rohde 1993, Angot and Brasseur 1995) and affecting growth (Faisal and Imam 1990, Williams and Jones 1994, Scholz 1999). They can also be deleterious for cultured species (Scholz 1999, Kritsky and Heckmann 2002). Recently the local Fisheries Department of Funchal (Madeira Island, Portugal) initiated a program of adaptation of *P. bogaraveo* to culture conditions (Andrade *et al*. 2000). According to several authors, it is advisable to undertake studies of parasites and diseases of wild fishes before initiating commercial cultivation of the species, due to possible transferences of infections from wild to cultured individuals (McVicar 1997, Reno 1998). Taking this into account, a study of the helminth parasites of *P. bogaraveo* was done, concentrating on the occurrence of anisakid nematodes.

Materials and methods

Between November 1999 and July 2000, 77 blackspot seabreams, *Pagellus bogaraveo*, captured in Madeiran waters, Atlantic Ocean, were examined for the presence of nematodes. Fish were dissected and the visceral cavity and digestive tract examined for the presence of helminths with the aid

*Corresponding author: gcosta@uma.pt

of a Zeiss stereomicroscope. After dissection of the individuals, encapsulated or free nematode larvae from the visceral cavity and internal organs were collected and counted. Live nematodes were placed in tap water for relaxation, fixed in 70% ethanol, cleared in lactophenol and mounted in Entellan (Berland 1984). Morphological study of the nematode larvae was done using a Zeiss Axioplan photomicroscope equipped with DIC optics, and photographs were obtained by attached MC-Camera. Measurements of morphological features, were taken with the aid of an ocular micrometer, and given in mil-

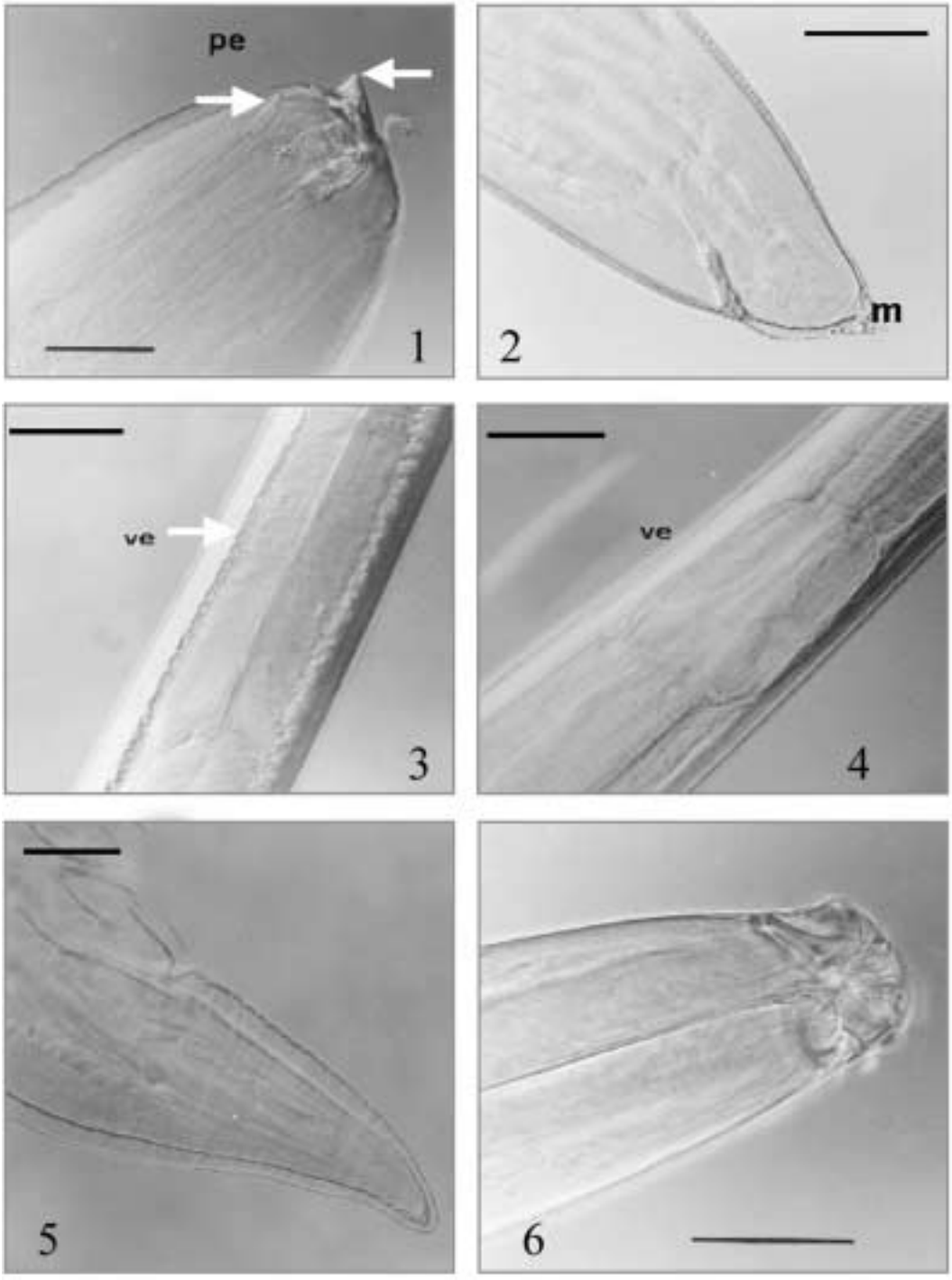

Fig. 1. Anterior end of *Anisakis simplex* s.l., Type I larva, from *Pagellus bogaraveo* showing excretory pore (pe) and boring tooth (arrowed). Scale bar = 100 µm. **Fig. 2.** Tail of *A. simplex* s.l., Type I larva showing mucron (m). Scale bar = 100 µm. **Fig. 3.** Ventricular region (ve) of *A. simplex* s.l., Type I larva. Scale bar = 200 µm. **Fig. 4.** Ventricular region (ve) of *A. simplex* s.l., Type II larva. Scale bar = 200 µm. **Fig. 5.** Tail of *A. simplex* s.l., Type II larva. Scale bar = 100 µm. **Fig. 6.** Anterior end of *Hysterothylacium* sp. (L4 stage) showing papillae. Scale bar = 50 µm

limeters or micrometers, (presented as means ± standard errors), with range (min.-max.). For histopathological study, samples of infected tissues were fixed in Bouin's fluid and subject to a standard histological protocol, to produce paraffin embedded samples suitable for sectioning and staining (Bancroft and Stevens 1990). Procedures for the molecular identification of the anisakid larvae followed those described in D'Amélio *et al*. (2000) and Pontes (2001). The condition factor (*K*) of each fish was calculated according to Fulton, as: K = weight (in g) × 1000/[total length (cm)]^3. Prevalence, intensity and abundance indices were calculated after Bush *et al*. (1997). Significance of relationships between condition factor and infection intensity of nematodes and of intensity of infection in relation to host length, were tested by Spearman correlation coefficient. Results were considered significant where $p<0.05$. Estimations of the level of aggregation of the anisakid larvae, were obtained by the calculations of the dispersion index (D), and the variance to mean ratio ($s^2/\times$), according to Poulin (1993), using the statistical package QP 1.0 (Rózsa *et al*. 2000).

Results

A total of 281 anisakid larvae in 69 fish were recovered during the study period (8 of the 77 fish were uninfected). A subsample of those larvae, composed of 94 individuals was retained for identification purposes. Seventy-nine larvae were identified as *Anisakis simplex* s.l., 50 of them being Type I larvae (Figs 1–3) and the remaining 29 Type II larvae (Figs 4 and 5) (sensu Berland 1961). Those larvae were found encapsulated in the external and internal walls of the stomach and mesenteries. Some of the *A. simplex* s.l. larvae were identified to the species level as *A. simplex* s.s. and *A. pegreffii* by the analysis of the rDNA intergenic spacers (ITS-1 and ITS-2). Total length of *Anisakis* Type I larvae was 21 ± 7 mm (6–32, n = 45) and Type II was 18 ± 2 mm (6–33, n = 22). Fifteen larvae, found in the intestine of the examined fish, were identified as *Hysterothylacium* sp. L3 and L4 stages (Figs 6–8). Molecular analysis of these larvae by PCR-RFLP of the rDNA region, ITS-1 and ITS-2 allowed the definition of restriction fragments typical of *Hysterothylacium*, but did not define the species. Length of these larvae was 13 ± 1 mm (10–16, n = 15), length of ventricular appendage was 1014 ± 74 µm (600–1280), length of the intestinal caecum was 148 ± 10 µm (100–210) and tail was 117 ± 41 µm (70–250).

Prevalence of the nematodes (both genera combined) was high (P = 89.61%, 95% confidence limits 0.8055 to 0.9541) and showed some differences with fish length from 81.3% (at the length class ≤ 25 cm) to 96.3% (at the length class ≥ 35 cm) (Table I). Intensity, in contrast, was low (mean intensity = 4.07, 95% bootstrap confidence limits 2.93 to 5.32), with mean values per length class of 3.5 ± 0.8 to 4.8 ± 2.0, with the majority of fish infected by only 1 or 2 parasites (n = 34). One fish was infected with 36 nematodes. A positive but not significant correlation was found between intensity and length (r_s = 0.0419, p = 0.717), and condition factor and intensity (r_s = 0.242, p = 0.035). The high values of prevalence and low values of intensity (except for 4 fish), may indicate that the anisakid larvae are dispersed throughout the host population, which was confirmed by the values of D = 0.534 and $s^2/\times$ = 7.354. No histopathological lesions were found associated with the presence of encapsulated nematodes in the stomach wall.

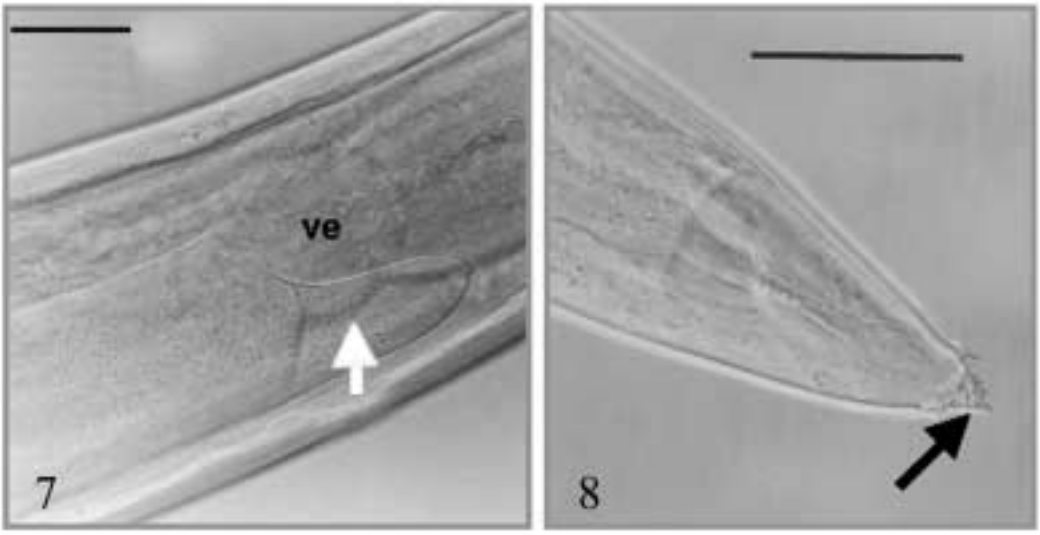

Fig. 7. Oesophagus region, ventriculus (ve) and intestinal caecum (arrowed) of *Hysterothylacium* sp. Scale bar = 62.5 µm. **Fig. 8.** Posterior end of *Hysterothylacium* sp. with cactus-tail. Scale bar = 100 µm

Table I. Prevalence, intensity and abundance of anisakid larvae in *Pagellus bogaraveo*, relative to fish length, from November 1999 to July 2000

Length class (cm)	No. fish examined	No. fish infected	Prevalence (%)	Parasites (min.-max.)	Mean intensity ± S.E.	Abundance ± S.E.
≤ 25	16	13	81.3	47 (1–10)	3.6 ± 0.8	2.9 ± 0.7
25–35	34	30	88.2	144 (1–36)	4.8 ± 2.0	4.2 ± 1.2
≥ 35	27	26	96.3	90 (1–17)	3.5 ± 0.8	3.3 ± 0.7

Discussion

Anisakid nematodes, both larvae and adults, are common parasites of a wide range of marine fish species (Smith and Wootten 1978, Anderson 1992, Williams and Jones 1994). The genus *Anisakis*, which occurs in fish at the larval stage, was previously reported infecting *P. bogaraveo* from the Atlantic (Berland 1961) and *P. erythrinus* from the Mediterranean (Petter and Maillard 1988). The species *A. simplex* s.s. and *A. pegreffii* were reported from the chub mackerel, *Scomber japonicus*, oceanic horse mackerel, *Trachurus picturatus*, and the black-scabbard fish, *Aphanopus carbo* from Madeiran waters (Pontes 2001, Costa *et al*. 2003). For *A. simplex* s.s. the intermediate/paratenic hosts are mainly benthic and demersal (Mattiucci *et al*. 1997), which is consistent with their occurrence in *P. bogaraveo*. Both *A. simplex* and *A. pegreffii* are characterized by larvae of Type I (sensu Berland 1961). Several larvae of Type II were also recovered during this study, which were not identified by molecular methods. Type II larvae are known to belong to *A. physeteris* and *A. brevispiculata* (Mattiucci *et al*. 1986, 2001) and have been reported in other fishes from Madeiran waters (Pontes 2001, Costa *et al*. 2003). In addition, *A. physeteris* is typical of epipelagic intermediate/paratenic hosts of the Mediterranean and Atlantic Ocean, while *A. brevispiculata* appears to be distributed in central and southern Atlantic waters. Its final host *Kogia breviceps* (pigmy sperm whale) has been reported in Madeiran waters by Mathias (1988). Thus at least one further *Anisakis* species occur in *P. bogaraveo*, either *A. physeteris* or *A. brevispiculata* or both. Further molecular work is required to clarify the situation.

Anisakid nematodes of the genus *Hysterothylacium* use fish as both intermediate and definitive hosts, in which they attain maturity. Some *Hysterothylacium* species were reported from sparid fishes, namely *Pagellus acarne* (Petter and Cabaret 1995) and a related species *Diplodus sargus* from the Mediterranean (Petter and Maillard 1988). The morphology of the *Hysterothylacium* larvae in the present work was compared with those of *Hysterothylacium reliquens* and *H. fabri*, as these species were reported to occur either in the genus *Pagellus* or in sparid members in general (Norris and Overstreet 1975, Petter and Maillard 1988, Petter and Cabaret 1995). In particular, measurements of the total body length, length of ventricular appendage, the intestinal caecum and tail, in *P. bogaraveo* specimens in the present work (10–16 mm and 600–1280, 100–210, 80–265 µm) closely approximated those given for *H. fabri* found in *Diplodus sargus* (sparid) and other fish species from the Mediterranean (10.8–16 mm and 550–1275, 170, 70–250 µm) (Petter and Maillard 1988).

Moreover, in a survey of parasites from the Turkish-wrasse, *Thalassoma pavo* (Labridae) in Madeira during 1999, L3 stages of *Hysterothylacium* sp. were found with very long ventricular appendage about 6.8 times bigger than the intestinal caecum. This fits in well with the description of Petter and Maillard (1988) in which is reported the occurrence of *H. fabri* also in *T. pavo*. Tentative molecular identification of some of the larval *Hysterothylacium* sp. found in the blackspot seabream could only assign larvae to the genus *Hysterothylacium*, as no adult forms were available. Molecular studies of members of this genus to date were only done for *H. aduncum*. Our species did not correspond to *H. aduncum* based on the molecular results. It would thus be interesting in future research work to look for adult stages of *Hysterothylacium* in Madeiran fishes to allow a more detailed characterization of molecular markers appropriate for distinguishing members of this genus.

Histopathological analysis of the effect of the anisakid larvae in the infected tissues, showed no specific lesions due to infection with nematodes. However, the pathological effect depends on the number of larvae present and the size (age) of the fish (Lester and Adams 1974, Rohde 1984). Pavanelli *et al*. (1998) stated that in juvenile fish, nematode infections may lead to some mortality. Apart from mortalities, the presence of encapsulated larvae in fish tissues may lead to devaluation of the quality of the fish (Rohde 1993, Angot and Brasseur 1995) which is rather important in aquaculture. It is also worthwhile to mention that caged fish may be fed on fish meat, which could contain nematode larvae, thus becoming infected. On the other hand, if caged fish are reared in open sea cages, as it is done in Madeira, they may ingest the anisakid intermediate hosts, such as pelagic crustaceans (Smith 1983, Marcogliese 1995), and thus become infected by these nematodes. It would also be worthwhile to look for anisakids in the muscle tissue in future parasitological investigations of this fish species.

Prevalence of infection was high and intensity low (except in 4 of the fish examined), a picture already found for other fish hosts in Madeira, namely *S. japonicus* and *T. picturatus* (Costa *et al*. 2003). Since anisakids are not host specific at the

larval stage they may be found in a wide range of different available fish host species, and this may result in a higher probability of transmission (Smith 1983, Mattiucci *et al*. 1997).

Acknowledgements. Financial support for the present study was partially provided by Portuguese Foundation for Science and Technology (FCT/POCTI/Lisbon) through their pluriannual programs. The authors are grateful to Dr. Ken MacKenzie (Department of Zoology, University of Aberdeen, UK) for his helpful comments and English corrections.

References

Anderson R.C. 1992. Nematode parasites of vertebrates: their development and transmission. CAB International, Wallingford.

Andrade C.A.P., Gouveia N.M.A., Gomes J.R.J., Timóteo V.M.F.A. 2000. Preliminary results of blackspot seabream (*Pagellus bogaraveo*) culture under offshore conditions. Does inappropriate feed impair growth? *The Ninth International Symposium on Nutrition & Feeding in Fish*, *21–25 May*, *Miyazaki*, *Japan*.

Angot V., Brasseur P. 1995. Les larves d'anisakidés et leur incidence sur la qualité des poissons et produits de poisson. *Revue de Medecine Véterinaire*, 146, 791–804.

Bancroft J.D., Stevens A. 1990. Theory and practice of histological techniques. Churchill Livingstone, London.

Bauchot M.L., Hureau J.C. 1986. Sparidae. In: *Fishes of the northeastern Atlantic and the Mediterranean*. Vol. II (Eds. P.J.P. Whitehead, M.L. Bauchot, J.C. Hureau, J. Nielsen and E. Tortonese). UNESCO, UK, 883–907.

Berland B. 1961. Nematodes from some Norwegian marine fishes. *Sarsia*, 2, 1–50.

Berland B. 1984. Basic techniques involved in helminth preservation. *Systematic Parasitology*, 6, 242–245.

Bush A.O., Lafferty K.D., Lotz J.M., Shostak A.W. 1997. Parasitology meets ecology on its own terms: Margolis *et al*. revisited. *Journal of Parasitology*, 83, 575–583.

Costa G., Pontes T., Mattiucci S., D'Amélio S. 2003. The occurrence and infection dynamics of *Anisakis* larvae in the black-scabbard fish, *Aphanopus carbo*, chub mackerel, *Scomber japonicus*, and oceanic horse mackerel, *Trachurus picturatus* from Madeira, Portugal. *Journal of Helminthology*, 77, 163–166.

D'Amélio S., Mathiopoulos K.D., Santos C.P., Pugachev O.N., Webb S.C., Picanço M., Paggi L. 2000. Genetic markers in ribosomal DNA for the identification of members of the genus *Anisakis* (Nematoda: Ascaridoidea) defined by polymerase chain reaction-based restriction fragment length polymorphism. *International Journal for Parasitology*, 30, 223–226.

Faisal M., Imam E.A. 1990. *Microcotyle chrysophrii* (Monogenea: Polyopisthocotylea), a pathogen for culture and wild sea bream, *Sparus aurata*. In: *Pathology in marine science* (Eds. F. Perkins and C. Cheng). Academic Press, New York, 283–290.

Iversen E.S. 1996. Living marine resources: their utilization and management. Chapman & Hall, New York.

Kritsky D.C., Heckmann R. 2002. Species of *Dactylogyrus* (Monogenoidea: Dactylogyridae) and *Trichodina mutabilis* (Ciliata) infesting koi carp, *Cyprinus carpio*, during mass mortality at a commercial rearing facility in Utah, U.S.A. *Comparative Parasitology*, 69, 217–218.

Lester R.J.G., Adams J.R. 1974. *Gyrodactylus alexanderi*: reproduction, mortality, and effect on its host *Gasterosteus aculeatus*. *Canadian Journal of Zoology*, 52, 827–833.

Lom J., Dyková D. 1992. Protozoan parasites of fishes. Developments in aquaculture and fisheries science. Vol. 26. Elsevier, London.

Marcogliese D.J. 1995. The role of zooplankton in the transmission of helminth parasites to fish. *Reviews in Fish Biology and Fisheries*, 5, 336–371.

Mathias M.L. 1988. An annotated list of the mammals recorded from the Madeira Islands. *Boletim do Museu Municipal do Funchal*, 40, 111–137.

Mattiucci S., Nascetti G., Bullini L., Orecchia P., Paggi L. 1986. Genetic structure of *Anisakis physeteris* and its differentiation from the *Anisakis simplex* complex (Ascaridida: Anisakidae). *Parasitology*, 93, 383–387.

Mattiucci S., Nascetti G., Cianchi R., Paggi L., Arduino P., Margolis L., Brattey J., Webb S.C., D'Amélio S., Orecchia P., Bullini L. 1997. Genetic and ecological data on the *Anisakis simplex* complex with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). *Journal of Parasitology*, 83, 401–416.

Mattiucci S., Paggi L., Nascetti G., Abollo E., Webb S.C., Pascual S., Cianchi R., Bullini L. 2001. Genetic divergence and reproductive isolation between *Anisakis brevispiculata* and *Anisakis physeteris* (Nematoda: Anisakidae). *International Journal for Parasitology*, 31, 9–14.

McVicar A.H. 1997. Interactions of pathogens in aquaculture with wild fish populations. *Bulletin of the European Association of Fish Pathologists*, 17, 197–200.

Mo T. 1994. Status of *Gyrodactylus salaris* problems and research in Norway. In: *Parasitic diseases of fish* (Eds. A.W. Pike and J.W. Lewis). Samara Publishers, Tresaith, Dyfed, UK, 43–56.

Molnár K., Székely C., Baska F. 1993. Mass mortality of eel in Lake Balaton due to *Anguillicola crassus* infection. *Bulletin of the European Association of Fish Pathologists*, 11, 211–212.

Norris D.E., Overstreet R.M. 1975. *Thynnascaris reliquens* sp. n. and *T. habena* (Linton, 1900) (Nematoda: Ascaridoidea) from fishes in the northern gulf of Mexico and eastern U.S. Seaboard. *Journal of Parasitology*, 61, 330–336.

Pavanelli G.C., Eiras J.C., Takemoto R.M. 1998. Doenças de peixes: profilaxia, diagnóstico e tratamento. Nupélia, Maringá, Brasil.

Petter A.J., Cabaret J. 1995. Ascaridoid nematodes of teleostean fishes from the eastern North Atlantic and seas of the North of Europe. *Parasite*, 2, 217–230.

Petter A.J., Maillard C. 1988. Larves d'ascarides parasites de poissons en Mediterranée occidentale. *Bulletin du Muséum d'Histoire Naturalles*, *Paris*, *Sér*. 4, *Sect*. *A*, 2, 347–369.

Pontes T.M.T. 2001. Molecular characterization of larval anisakid nematodes by PCR-based approaches. Diploma Thesis, Department of Biology, University of Madeira.

Poulin R. 1993. The disparity between observed and uniform distributions: a new look at the parasite aggregation. *International Journal for Parasitology*, 23, 937–944.

Reno P.W. 1998. Factors involved in the dissemination of disease in fish populations. *Journal of Aquatic Animal Health*, 10, 160–171.

Rohde K. 1984. Diseases caused by metazoans: Helminths. In: *Diseases of marine animals*. Vol. IV. Part 1. *Introduction*, *Pisces* (Ed. O. Kinne). Biologische Anstalt Helgoland, Hamburg, 193–320.

Rohde K. 1993. Ecology of marine parasites. CAB International, Wallingford.

Rózsa L., Reiczigel J., Majoros G. 2000. Quantifying parasites in samples of hosts. *Journal of Parasitology*, 86, 228–232.

Scholz T. 1999. Parasites in cultured and feral fish. *Veterinary Parasitology*, 84, 317–335.

Smith J.W. 1983. *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878) (Nematoda: Ascaridoidea): Morphology and morphometry of larva from euphausiids and fish, and a review of the

life-history and the ecology. *Journal of Helminthology*, 57, 205–224.
Smith J.W., Wootten R. 1978. *Anisakis* and anisakiasis. *Advances in Parasitology*, 16, 93–163.
Williams H.H., Jones A. 1994. Parasitic worms of fish. Taylor & Francis, London.

(Accepted March 4, 2004)