

Low diversity of helminth parasites in *Sardina pilchardus* and *Engraulis encrasicolus* (Clupeidae) from the Bay of Biscay

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Abstract. Parasitological data are increasingly used to provide information on host populations, trophic interactions and free-living biodiversity. In the present study, we investigated parasitic helminths in *Sardina pilchardus* and *Engraulis encrasicolus* from the Bay of Biscay (north-east Atlantic). In addition, helminths infecting *E. encrasicolus* were reviewed. Surprisingly, the field study revealed the occurrence of only three species of Ascaridoidea (*Anisakis simplex* s.s., *Anisakis pegreffii* and *Hysterothylacium aduncum*) in both fish species despite the high diversity of helminth parasites (mostly heteroxenous trophically transmitted) recorded throughout their distribution range (i.e. 39 and 15 taxa for *S. pilchardus* and *E. encrasicolus* respectively, with 12 shared taxa among them). This low diversity of helminth parasites in the Bay of Biscay may be indicative of a low free-living biodiversity. Moreover, similarities in the helminth parasites of *S. pilchardus* and *E. encrasicolus*, recorded both in the present field study and across their entire geographic distribution, suggest ecological and feeding similarities between these two planktivorous fish. However, the higher prevalence and mean intensity of Ascaridoidea in *E. encrasicolus* (v. *S. pilchardus*) in the Bay of Biscay also highlighted differences that were considered the result of its higher trophic plasticity.

Additional keywords: anchovy, Ascaridoidea, feeding ecology, low biodiversity, sardine, small pelagic fish.

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Introduction

Small pelagic fish, such as *Sardina pilchardus* and *Engraulis encrasicolus*, contribute up to 50% of the total landing of marine species worldwide and play a considerable role in connecting the lower and upper trophic levels (for reviews, see Fréon *et al.* 2005; Palomera *et al.* 2007; Morello and Arneri 2009). First, they are both suspension and raptorial feeders, and their flexible, dual planktivorous diet includes diverse taxa, such as crustaceans of various sizes (copepods, cladocerans, euphausiids, amphipods and decapods), appendicularians and fish eggs and larvae, depending on the season and fish size (Bacha and Amara 2009; Morello and Arneri 2009; Petitgas 2010; Atkinson *et al.* 2014; Chouvelon *et al.* 2014, 2015). Second, they are preyed on by many predator species, such as planktonic chaetognaths (as larval fish), demersal and medium-to-large pelagic fish, marine mammals and seabirds (Morello and Arneri 2009; Petitgas 2010; Certain *et al.* 2011). Consequently, *S. pilchardus*

is the host for a great number of parasites, including 39 helminth taxa, most of which are trophically transmitted (for a review, see Marzoug *et al.* 2012). In the same way, parasites of *E. encrasicolus* are probably highly diverse.

Helminth parasites are increasingly used to provide information on host populations, potentially helping manage fish stocks, free-living biodiversity and changes in ecosystem structure and functioning (for reviews, see MacKenzie 2002; Marcogliese 2004).

In the present study, helminth parasites were investigated in adults of *S. pilchardus* and *E. encrasicolus* sampled in different sites across the Bay of Biscay (north-east Atlantic). In addition, helminth parasites of *E. encrasicolus* have been reviewed across their entire geographic distribution. The results are discussed in relation to the potential role of helminth parasites as indicators of food webs, free-living biodiversity and ecosystem stress.

Materials and methods

Study site and samplings

PELGAS (PELAgique GAScogne) is an annual pelagic acoustic spring survey that has been led by the French Research Institute for Exploitation of the Sea (IFREMER) since 2000 in the Bay of Biscay. It aims to monitor the abundance of small pelagic fish in

order to help conserve fish stocks. In spring 2012, PELGAS provided 49 *S. pilchardus* and 52 *E. encrasicolus* collected by pelagic trawling (for 30–45 min) at various locations and depths in the survey area (43°30'–49°00'N, 1°10'–6°00'W; Fig. 1; Table 1). All fish were at an adult stage according to their age determined by otolithometry (International Council for the Exploration of the Sea, ICES 2005, 2010); mean (±CL) age

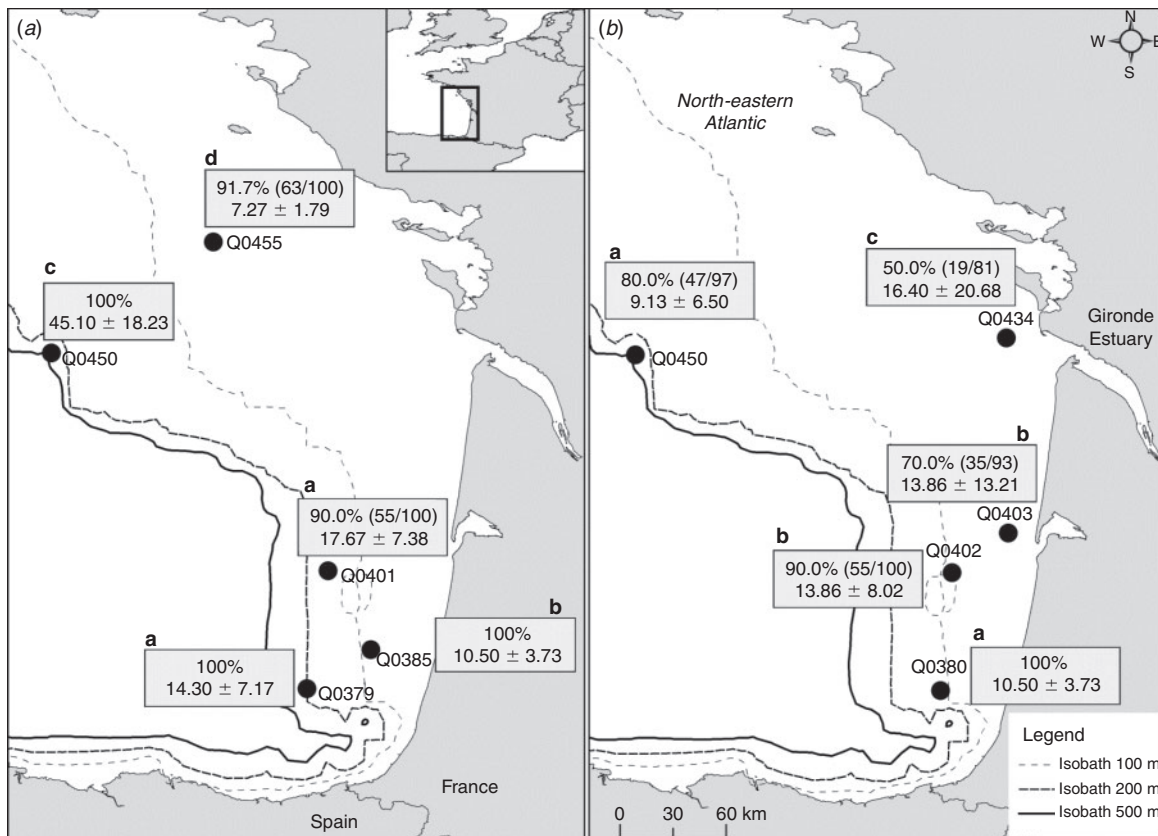


Fig. 1. Percentage prevalence (±95% confidence level) and mean intensity (±95% CL) of Ascaridoidea in (a) *Engraulis encrasicolus* and (b) *Sardina pilchardus* sampled in spring 2012 across the Bay of Biscay. Inter-site differences in the prevalence for each fish species were not significant ($P > 0.05$). Significant differences in mean intensities among sites for each fish species are indicated by different letters (a, b, c, d).

Table 1. Geographical position (latitude and longitude) and water depth of trawls, and the number and mean total length (TL ± 95% CL) of *Sardina pilchardus* and *Engraulis encrasicolus* sampled in spring 2012 across the Bay of Biscay
CL, confidence limit

	Trawls	Latitude	Longitude	Depth (m)	Number sampled	TL ± 95% CL (cm)
<i>S. pilchardus</i> (n = 49)	Q0380	43°52'12"N	1°44'24"W	119.2	9	20.62 ± 0.61
	Q0402	44°28'12"N	1°40'48"W	108.7	10	21.05 ± 0.48
	Q0403	40°10'12"N	1°23'60"W	45.5	10	21.65 ± 0.56
	Q0434	45°39'36"N	1°24'36"W	23.3	10	21.25 ± 0.82
	Q0450	45°34'12"N	3°17'60"W	157.2	10	21.40 ± 0.53
<i>E. encrasicolus</i> (n = 52)	Q0379	43°51'36"N	1°59'24"W	140.5	10	16.40 ± 0.82
	Q0385	44°04'12"N	1°40'12"W	112.7	10	15.76 ± 0.49
	Q0401	44°28'12"N	1°53'24"W	129.6	10	18.22 ± 1.99
	Q0450	44°34'12"N	3°17'60"W	157.2	10	17.05 ± 0.27
	Q0455	46°07'48"N	2°28'12"W	79.3	12	12.27 ± 0.67

1.76 ± 0.06 years for *S. pilchardus* and 1.92 ± 0.04 years for *E. encrasicolus*).

Parasitological research

All fish were measured (cm) and frozen before complete examination of helminth parasites, as in previous studies (Gérard *et al.* 2013, 2015). All organs and tissues (skin, muscles, eyes, brain, gills, heart, body cavity, digestive system, gonads and swim bladder) were meticulously dissected under a binocular stereomicroscope. All helminth parasites found were numbered. The identification of the nematodes (i.e. Ascaridoidea) was based first on morphological criteria according to Fagerholm (1991) and Anderson *et al.* (2009), demonstrating the occurrence of two genera (i.e. *Anisakis* and *Hysterothylacium*). Second, all *Anisakis* ($n = 75$) and some *Hysterothylacium* ($n = 10$) specimens preserved in alcohol (70%) were subjected to molecular identification at the species level via DNA sequencing (cytochrome oxidase subunit 2 (*cox2*)). Total DNA of parasites was extracted from all individual nematode tissues using the ChargeSwitch Forensic DNA Purification Kit (Invitrogen, Carlsbad, CA, USA). A 530-bp fragment of the *cox2* gene was amplified in collaboration with the French National Research Agency (ANR) Fish-Parasites project (D. C. Eduardo, unpubl. data). Automated DNA sequencing was performed by Genoscreen (Lille, France) and sequences were then analysed using BioEdit software (Hall 1999) and compared with nucleotide sequences from GenBank (National Center for Biotechnology Information) using BLAST.

The parasite descriptors used were: (1) prevalence, calculated as the number of hosts infected with a particular parasite taxon divided by the number of hosts examined; and (2) mean intensity, which is the total number of parasites of a particular species found in a sample divided by the number of hosts infected with that parasite (Bush *et al.* 1997).

Statistical analyses

Statistical analyses were made using R-Cran project free software (R Core Team 2014). Differences were considered significant at two-tailed $P < 0.05$. Data are reported as the mean \pm 95% confidence limits (CL).

General linear models (GLMs; McCullagh 1984) followed by analysis of variance (ANOVA; Chi-Square test) were used to check the significance of differences between *S. pilchardus* and *E. encrasicolus* in terms of the prevalence (binomial model) and mean intensity (Poisson model) of parasites (Hastie and Pregibon 1992). Then, inter-site comparisons were made by successively modifying the reference site in the GLMs.

Results

Helminth parasites of *S. pilchardus* and *E. encrasicolus* in the Bay of Biscay

All parasites recovered were nematodes belonging to the super family Ascaridoidea (*Anisakis* and *Hysterothylacium* genera) at the third larval stage (L3) in the body cavity or adults in the digestive system. No helminth parasites were detected in other organs. The overall prevalence of helminth parasites was 77.6% (95% CL 64–88) for *S. pilchardus* ($n = 49$) and 96.2% (95% CL 87–100) for *E. encrasicolus* ($n = 52$), with a mean intensity of

11.92 ± 4.21 and 18.76 ± 5.61 respectively. According to the DNA sequences (*cox2*) and their comparison with sequences present in the GenBank nucleotide database, three parasite species were unambiguously identified in both fish species: *Anisakis simplex s.s.* (Anisakidae), *Anisakis pegreffi* (Anisakidae) and *Hysterothylacium aduncum* (Raphidascarididae). Of the 466 nematodes found in *S. pilchardus*, 99.4% were *H. aduncum*, 0.4% were *A. simplex s.s.* and 0.2% were *A. pegreffi*. Of the 982 nematodes found in *E. encrasicolus*, 92.3% were *H. aduncum*, 5.7% were *A. simplex s.s.* and 1.7% were to *A. pegreffi*.

The overall prevalence and mean intensity of Ascaridoidea were significantly higher in *E. encrasicolus* than *S. pilchardus* ($P = 0.01$ and $P = 2 \times 10^{-15}$ respectively). In the site common to the two fish species (Q450), the mean intensity was also significantly higher for *E. encrasicolus* than *S. pilchardus* ($P = 5 \times 10^{-16}$), but prevalence did not differ significantly (Fig. 1).

Inter-site differences in the prevalence of Ascaridoidea were not significant for either fish species (Fig. 1). However, significant differences occurred between some sites in the mean intensity of parasites for both *S. pilchardus* and *E. encrasicolus* (Fig. 1).

Checklist of helminth parasites recorded in *E. encrasicolus*

Only two complete parasitological investigations of *E. encrasicolus* were found in the literature, namely the study of Reichenbach-Klinke (1958) on 100 individuals from the western Mediterranean (Italy) and that of Shukhgalter (2002) on 290 individuals from the north-eastern Atlantic (north-western coast of Africa (Morocco and Sahara)). In all, 15 helminth taxa were recorded in *E. encrasicolus* (Table 2), including the Ascaridoidea species found in the present field study. All the taxa (except monogenean *Pseudoanthocotyle* sp.) were heteroxenous and trophically transmitted, with at least three hosts in their life cycle, including invertebrates and vertebrates. Twelve of the 15 helminth taxa listed in *E. encrasicolus* also infected *S. pilchardus* (Table 2).

Discussion

Similarities in the helminth parasites of *S. pilchardus* and *E. encrasicolus* recorded in both the present field study (three species of Ascaridoidea in the Bay of Biscay) and across their entire geographic distribution (12 trophically transmitted taxa in common among Digenea, Cestoda, Nematoda and Acanthocephala) highlight feeding similarities. Such similarities have been demonstrated previously between these two small planktivorous pelagics using analyses of stomach contents and stable isotopes despite a greater trophic plasticity for *E. encrasicolus* (for reviews, see Morello and Arneri 2009; Chauvelon *et al.* 2014, 2015). In the Bay of Biscay, *S. pilchardus* and *E. encrasicolus* mainly feed with small- to medium-sized copepods (e.g. *Acartia* sp. and *Temora* sp.; Chauvelon *et al.* 2014, 2015). These copepods probably act as intermediate hosts for the Ascaridoidea recorded herein, with *S. pilchardus* and *E. encrasicolus* serving as trophic links in the life cycles of both *Anisakis* and *Hysterothylacium* (Køie 1993; Mattiucci and Nascetti 2008; Kijewska *et al.* 2009). More precisely, these small pelagic fish are paratenic hosts for *A. simplex s.s.* and *A. pegreffi*, harbouring L3 in their body cavity (Mattiucci

Table 2. Checklist of the helminth parasite taxa ($n = 15$) recorded in *Engraulis encrasicolus*
BS, Baltic Sea; NEA, north-east Atlantic; WM, west Mediterranean

Parasites	Area	References
Monogenea		
Family Mazocraeidae		
<i>Pseudoanthocotyle</i> sp.	NEA	Shukhgalter (2002)
Digenea		
Family Acanthocolpidae		
<i>Stephanostomum</i> sp. (metacercariae) ^A	NEA	Shukhgalter (2002)
Family Bucephalidae		
<i>Bucephalus</i> sp. (metacercariae) ^A	WM	Reichenbach-Klinke (1958)
Family Faustulidae		
<i>Bacciger bacciger</i> ^A	NEA	Shukhgalter (2002)
<i>Pseudobacciger harengulae</i> ^A	BS	Dimitrov et al. (1999)
Family Hemiuridae		
<i>Aphanurus stossichii</i> ^A	NEA, WM	Reichenbach-Klinke (1958), Shukhgalter (2002)
<i>Aphanurus virgula</i> ^A	BS	Kostadinova et al. (2004)
Family Lecithasteridae		
<i>Aponurus lagunculus</i>	WM	Reichenbach-Klinke (1958)
<i>Lecithaster confusus</i> ^A	NEA	Shukhgalter (2002)
Cestoda		
Family Lacistorhynchidae		
<i>Lacistorhynchus</i> sp. (plerocercoid)	NEA	Shukhgalter (2002)
Family Tetraphyllidae		
<i>Scolex pleuronectis</i> (plerocercoid) ^A	NEA, WM	Reichenbach-Klinke (1958), Shukhgalter (2002)
Nematoda		
Family Anisakidae		
<i>Anisakis simplex</i> s.l. (larvae) ^A	NEA	Shukhgalter (2002)
<i>Anisakis pegreffii</i> (larvae) ^A	WM	Piras et al. (2014)
Family Raphidascarididae		
<i>Hysterothylacium aduncum</i> ^A	NEA, WM	Reichenbach-Klinke (1958), Shukhgalter (2002)
Acanthocephala		
Family Rhadinorhynchidae		
<i>Rhadinorhynchus</i> sp. ^A	NEA	Shukhgalter (2002)

^ATaxa ($n = 12$) also recorded in *Sardina pilchardus* (Marzoug et al. 2012).

and Nascetti 2008), and both paratenic hosts (L3 in the body cavity) and definitive hosts (adults in the digestive system) for *H. aduncum* (Køie 1993). The higher prevalence and mean intensity of Ascaridoidea in *E. encrasicolus* than *S. pilchardus* in the Bay of Biscay (overall and in the common site Q450) can be explained, in part, by the greater trophic plasticity of *E. encrasicolus* foraging on a wider range of prey sizes compared with the diet of *S. pilchardus*, which is more limited to mesozooplanktonic species and potentially includes phytoplankton (Chouvelon et al. 2014, 2015). Inter-site differences in the mean intensity of Ascaridoidea recorded for each fish species may also suggest local variations in the already described feeding strategies and trophic resources used depending on depth and food availability, as shown through analyses of stomach contents and stable isotopes (for reviews, see Morello and Arneri 2009; Chouvelon et al. 2014, 2015). One can expect that spatial and temporal variations in resource availability and in the foraging strategy of small pelagic fish induce variations in their patterns of trophically transmitted parasites.

The low diversity of helminth parasites found in both fish species studied from the Bay of Biscay (three species of Ascaridoidea) is surprising compared with the 39 taxa for *S. pilchardus* and the 15 taxa for *E. encrasicolus* recorded across

their distribution range (Marzoug et al. 2012; Table 2). For example, 10 and eight taxa of helminth parasites were found in *S. pilchardus* and *E. encrasicolus* respectively from the north-eastern Atlantic (north-western coast of Africa) by Shukhgalter (2002). Because the diversity of helminth parasites is positively correlated with host diversity, it is increasingly considered to reflect an ecosystem's health and the potential degradation of environmental conditions (e.g. Marcogliese 2004, 2005; Hudson et al. 2006; Hechinger et al. 2007; Thieltges et al. 2011). In the Bay of Biscay, long-term investigations have shown that marine ecosystems are increasingly constrained by the effects of anthropogenic activities (e.g. pollution and over-fishing) and oceanoclimatic changes (e.g. water warming and increased stratification) that could threaten the biodiversity in both coastal and pelagic systems (Blanchard et al. 2006; Hémerly et al. 2008; Chust et al. 2011). Indeed, changes in fish assemblages linked to interacting fishing and climatic factors result in the increasing abundance of subtropical fish species (Blanchard et al. 2006). Overall, boreal species of vertebrates (fish, seabirds and marine mammals) with affinities for cold temperate waters declined significantly or even disappeared totally, whereas meridional vertebrates with affinities for hotter waters increased in population size (Hémerly et al. 2008).

Thus, the low diversity of helminth parasites in *S. pilchardus* and *E. encrasicolus* in the Bay of Biscay may be indicative of changes in free-living diversity with a loss of host diversity. Further extensive investigations on both parasite and free-living diversity in the Bay of Biscay are needed to assess the potential use of helminth parasites as indicators of changes in biocenosis and of stressed marine ecosystems.

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References

- Anderson, R. C., Chabaud, A. G., and Willmott, S. (Eds) (2009). ‘Keys to the Nematode Parasites of Vertebrates.’ (CABI: New York.)
- Atkinson, A., Hill, S. L., Barange, M., Pakhomov, E. A., Raubenheimer, D., Schmidt, K., Simpson, S. J., and Reiss, C. (2014). Sardine cycles, krill declines, and locust plagues: revisiting ‘wasp-waist’ food webs. *Trends in Ecology & Evolution* **29**, 309–316. doi:10.1016/J.TREE.2014.03.011
- Bacha, M., and Amara, R. (2009). Spatial, temporal and ontogenetic variation in diet of anchovy (*Engraulis encrasicolus*) on the Algerian coast (SW Mediterranean). *Estuarine, Coastal and Shelf Science* **85**, 257–264. doi:10.1016/J.ECSS.2009.08.009
- Blanchard, F., Thébaud, O., Guyader, O., Lorange, P., Boucher, J., and Chevaillier, P. (2006). Effets de la pêche et du réchauffement climatique sur la coexistence spatiale des espèces de poissons du golfe de Gascogne. Conséquences pour les pêcheries. IFREMER, Programme de recherche «Biodiversité et Changement Global». Available at <http://archiver.ifremer.fr/doc/00000/6347/> [Verified 29 September 2014].
- Bush, A. O., Lafferty, K. D., Lotz, J. M., and Shostak, A. W. (1997). Parasitology meets ecology on its terms: Margolis *et al.* revisited. *Journal of Parasitology* **83**, 575–583. doi:10.2307/3284227
- Certain, G., Masse, J., Van Canneyt, O., Petitgas, P., Doremus, G., Santos, M. B., and Ridoux, V. (2011). Investigating the coupling between small pelagic fish and marine top predators using data collected from ecosystem-based surveys. *Marine Ecology Progress Series* **422**, 23–39. doi:10.3354/MEPS08932
- Chouvelon, T., Chappuis, A., Bustamante, P., Lefebvre, S., Mornet, F., Guillou, G., Violamer, L., and Dupuy, C. (2014). Trophic ecology of European sardine *Sardina pilchardus* and European anchovy *Engraulis encrasicolus* in the Bay of Biscay (north-east Atlantic) inferred from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of fish and identified mesozooplanktonic organisms. *Journal of Sea Research* **85**, 277–291. doi:10.1016/J.SEAES.2013.05.011
- Chouvelon, T., Violamer, L., Dessier, A., Bustamante, P., Mornet, F., Pignon-Mussaïd, C., and Dupuy, C. (2015). Small pelagic fish feeding patterns in relation to food resource variability: an isotopic investigation for *Sardina pilchardus* and *Engraulis encrasicolus* from the Bay of Biscay (north-east Atlantic). *Marine Biology* **162**, 15–37. doi:10.1007/S00227-014-2577-5
- Chust, G., Borja, A., Caballero, A., Irigoien, X., Sáenz, J., Moncho, R., Marcos, M., Liria, P., Hidalgo, J., Valle, M., and Valencia, V. (2011). Climate change impacts on coastal and pelagic environments in the southeastern Bay of Biscay. *Climate Research* **48**, 307–332. doi:10.3354/CR00914
- Dimitrov, G. I., Bray, R. A., and Gibson, D. I. (1999). A redescription of *Pseudobacciger harengulae* (Yamaguti, 1938) (Digenea: Faustulidae) from *Sprattus sprattus phalericus* (Risso) and *Engraulis encrasicolus ponticus* Alexandrov off the Bulgarian Black Sea coast, with a review of the genus *Pseudobacciger* Nahhas & Cable, 1964. *Systematic Parasitology* **43**, 133–146. doi:10.1023/A:1006166502924
- Fagerholm, H.-P. (1991). Systematic implications of male caudal morphology in ascaridoid nematode parasites. *Systematic Parasitology* **19**, 215–229. doi:10.1007/BF00011888
- Fréon, P., Cury, P., Shannon, L., and Roy, C. (2005). Sustainable exploitation of small pelagic fish stocks challenged by environmental and ecosystem changes: a review. *Bulletin of Marine Science* **76**, 385–462.
- Gérard, C., Amilhat, E., Trancart, T., Faliex, E., Virag, L., Feunteun, E., and Acou, A. (2013). Influence of introduced vs. native parasites on the body condition of migrant silver eels. *Parasite* **20**, 38. doi:10.1051/PARASITE/2013040
- Gérard, C., Verrez-Bagnis, V., Jérôme, M., and Lasne, E. (2015). *Petromyzon marinus* (Petromyzontidae), an unusual host for helminth parasites in Western Europe. *Diseases of Aquatic Organisms* **113**, 263–267. doi:10.3354/DAO02842
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium* **41**, 95–98.
- Hastie, T. J., and Pregibon, D. (1992). Generalized Linear Models. In ‘Statistical Models’. (Eds J. M. Chambers and T. J. Hastie.) pp. 195–247. (Wadsworth and Brooks/Cole: Pacific Grove, CA, USA.)
- Hechinger, R. F., Lafferty, K. D., Huspeni, T. C., Brooks, A. J., and Kuris, A. M. (2007). Can parasites indicate free-living diversity? Relationships between the species richness and abundance of larval trematodes with that of local benthos and fishes. *Oecologia* **151**, 82–92. doi:10.1007/S00442-006-0568-Z
- Hémery, G., D’Amico, F., Castege, I., Dupont, B., D’Elbée, J., Lalanne, Y., and Mouches, C. (2008). Detecting the impact of oceanic-climatic changes on marine ecosystems using a multivariate index: the case of the Bay of Biscay (North Atlantic–European Ocean). *Global Change Biology* **14**, 27–38.
- Hudson, P. J., Dobson, A. P., and Lafferty, K. D. (2006). Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology & Evolution* **21**, 381–385. doi:10.1016/J.TREE.2006.04.007
- ICES (2005). Report of the Workshop on sardine otolith age reading and biology. Lisbon, Portugal. Available at <http://www.ices.dk/community/Documents/PGCCDBS/pil.agewk2005.pdf> [Verified 15 December 2014].
- ICES (2010). ‘Report of the Workshop on Age Reading of European Anchovy (WKARA).’ ICES Cooperative Research Report 43. (International Council of the Exploration of the Sea: Sicily, Italy.)
- Kijewska, A., Dzido, J., Shukhgalter, O., and Rokicki, J. (2009). Anisakid parasites of fishes caught on the African shelf. *The Journal of Parasitology* **95**, 639–645. doi:10.1645/GE-1796.1
- Koie, M. (1993). Aspects of the life-cycle and morphology of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda, Ascaridoidea, Anisakidae). *Canadian Journal of Zoology* **71**, 1289–1296. doi:10.1139/Z93-178
- Kostadinova, A., Gibson, D. I., Balbuena, J. A., Power, A. M., Montero, F. E., Aydogdu, A., and Raga, J. A. (2004). Redescriptions of *Aphanurus stossichii* (Monticelli, 1891) and *A. virgula* Looss, 1907 (Digenea: Hemiuridae). *Systematic Parasitology* **58**, 175–184. doi:10.1023/B:SYPA.0000032928.22470.9E
- MacKenzie, K. (2002). Parasites as biological tags in population studies of marine organisms: an update. *Parasitology* **124**, S153–S163. doi:10.1017/S0031182002001518
- Marcogliese, D. J. (2004). Parasites: small players with crucial roles in the ecological theater. *EcoHealth* **1**, 151–164. doi:10.1007/S10393-004-0028-3
- Marcogliese, D. J. (2005). Parasites of the superorganism: are they indicators of ecosystem health? *International Journal for Parasitology* **35**, 705–716. doi:10.1016/J.IJPARA.2005.01.015

- Marzoug, D., Boutiba, Z., Gibson, D. I., Pérez-del-Olmo, A., and Kostadinova, A. (2012). Descriptions of digeneans from *Sardina pilchardus* (Walbaum) (Clupeidae) off the Algerian coast of the western Mediterranean, with a complete list of its helminth parasites. *Systematic Parasitology* **81**, 169–186. doi:10.1007/S11230-011-9335-6
- Mattiucci, S., and Nascetti, G. (2008). Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. *Advances in Parasitology* **66**, 47–148. doi:10.1016/S0065-308X(08)00202-9
- McCullagh, P. (1984). Generalized linear models. *European Journal of Operational Research* **16**, 285–292. doi:10.1016/0377-2217(84)90282-0
- Morello, E. B., and Ameri, E. (2009). Anchovy and sardine in the Adriatic Sea: an ecological review. *Oceanography and Marine Biology – an Annual Review* **47**, 209–256.
- Palomera, I., Olivar, M. P., Salat, J., Sabatés, A., Coll, M., García, A., and Morales-Nin, B. (2007). Small pelagic fish in the NW Mediterranean Sea: an ecological review. *Progress in Oceanography* **74**, 377–396. doi:10.1016/J.POCEAN.2007.04.012
- Petitgas, P. (2010). 'Life Cycle Spatial Patterns of Small Pelagic Fish in the Northeast Atlantic.' ICES Cooperative Research Report 306. (Alheit J.)
- Piras, M. C., Tedde, T., Garippa, G., Virgilio, S., Sanna, D., Farjallah, S., and Merella, P. (2014). Molecular and epidemiological data on *Anisakis* spp. (Nematoda: Anisakidae) in commercial fish caught off northern Sardinia (western Mediterranean Sea). *Veterinary Parasitology* **203**, 237–240. doi:10.1016/J.VETPAR.2014.02.003
- R Core Team (2014). R: a language and environment for statistical computing. (R Foundation for Statistical Computing: Vienna, Austria.) Available at <http://www.R-project.org/> [Verified 28 July 2015].
- Reichenbach-Klinke, H.-H. (1958). Les parasites de la sardine (*Sardina pilchardus* Walb.) et de l'anchois (*Engraulis encrasicolus* Rond.). *Rapport Commission Internationale de l'Exploration scientifique en Mer Méditerranée* **14**, 351–353.
- Shukhgalter, O. (2002). Ecological and faunistic analysis of *Sardina pilchardus* Walb. 1792 and *Engraulis encrasicolus* L. 1758 parasitofauna along the Northwest African coast. In 'Fisheries and Biological Research 2000–2001. Vol. 1. The Atlantic Ocean and Southeast Pacific'. pp. 126–133. (AtlantNIRO: Kaliningrad, Russia.)
- Thieltges, D. W., Hof, C., Dehling, D. M., Brändle, M., Brandl, R., and Poulin, R. (2011). Host diversity and latitude drive trematode diversity patterns in the European freshwater fauna: trematode diversity patterns. *Global Ecology and Biogeography* **20**, 675–682. doi:10.1111/J.1466-8238.2010.00631.X