Parasitology Open

cambridge.org/pao

Research Article

Cite this article: Couso-Pérez S, Cañizo-Outeiriño A, Campo-Ramos R, Ares-Mazás E, Gómez-Couso H (2018). Remarkable differences in the presence of the acanthocephalan parasite *Echinorhynchus truttae* in brown trout (*Salmo trutta*) captured in two adjacent river basins in Galicia (NW Spain). *Parasitology Open* **4**, e5, 1–4. https://doi.org/10.1017/pao.2017.24

Received: 6 October 2017
Revised: 20 December 2017
Accepted: 21 December 2017

Key words:
Echinorhynchus truttae; prevalence; *Salmo trutta*; Spain

Author for correspondence:
Hipólito Gómez-Couso, E-mail: hipolito.gomez@usc.es

Remarkable differences in the presence of the acanthocephalan parasite *Echinorhynchus truttae* in brown trout (*Salmo trutta*) captured in two adjacent river basins in Galicia (NW Spain)

Seila Couso-Pérez[1], Antonio Cañizo-Outeiriño[1], Rainer Campo-Ramos[1], Elvira Ares-Mazás[1] and Hipólito Gómez-Couso[1,2]

[1]Laboratory of Parasitology, Department of Microbiology and Parasitology, Faculty of Pharmacy, Campus Vida, University of Santiago de Compostela, 15782 Santiago de Compostela, A Coruña, Spain and [2]Institute of Food Research and Analysis, University of Santiago de Compostela, 15782 Santiago de Compostela, A Coruña, Spain

Abstract

This is the first report on the presence of acanthocephalan parasite *Echinorhynchus truttae* in brown trout (*Salmo trutta*) from Spain. A total of 343 fish were captured by local anglers from 19 rivers in the adjacent Tambre and Ulla watersheds in Galicia (north-western Spain). Macroscopic and microscopic analyses of the intestinal contents revealed the presence of adults and/or eggs of *E. truttae* in 53 of the 123 trout from Tambre river basin (43.1%). By contrast, parasitic forms of this acanthocephalan were only observed in eight of the 220 fish from Ulla basin (3.6%), showing significant differences between the prevalences obtained in two adjacent watersheds ($P < 0.001$, odds ratio 19.0). Prevalence was significantly higher in specimens >3 years (length >26.0 cm) than in younger specimens ($P < 0.05$). The absence of *Gammarus pulex* in the region suggests that native gammarid species in Galicia (*Echinogammarus lusitanicus* and *Echinogammarus beriyoni*) may act as intermediate host in the life cycle of *E. truttae*. Different prevalences of *E. truttae* indicate that the presence/abundance of the intermediate crustacean host may be different in the two river basins, probably as a consequence of various abiotic factors and anthropogenic activities.

Introduction

Rivers are continually undergoing changes that are closely linked to adjacent terrestrial ecosystems. Eutrophication of fluvial ecosystems as a result of anthropogenic activity (e.g. discharge of urban and industrial wastewaters and the use of fertilizers and pesticides in agriculture) leads to important changes in aquatic communities (zooplanktonic, benthic and fish communities) and loss of biodiversity (Gilbert and Avenant-Oldewage, 2017). Environmental conditions can also directly or indirectly affect the presence of parasitic organisms (mainly those parasites with complex life cycles) due to changes in the abundance and distribution of the respective intermediate and definitive hosts (Sures, 2004).

Echinorhynchus truttae Schrank, 1788, an acanthocephalan parasite of salmonids, is known to infect a variety of species, including brown trout (*Salmo trutta*), one of the most important species of freshwater fish in Europe and of high economic value. This parasite is found throughout Europe (including Ireland and the British Islands) and its range extends across Siberia to the Bering Strait (Wayland, 2013). The life cycle of *E. truttae* requires two hosts. Fish act as final hosts for the adult worms, which inhabit the digestive tract, and amphipods, such as *Gammarus*, act as the intermediate hosts that harbour the infective form (cystacanth). The life cycle is completed when the infected crustaceans are eaten by an appropriate definitive host (Crompton and Nickol, 1985; Kennedy, 2006).

During a large study of the parasitic fauna of brown trout captured in several rivers in north-western (NW) Spain, remarkable differences in the presence of *E. truttae* were observed. This work provided data on the prevalence, mean intensity and mean abundance of this acanthocephalan parasite in specimens of *S. trutta* captured in the adjacent Tambre and Ulla river basins in Galicia and constitutes the first report of this acanthocephalan in Spain.

Materials and methods

This study was carried out in 19 rivers belonging to Tambre ($n = 10$) and Ulla ($n = 9$) river basins, located on the Atlantic side of Galicia (NW Spain). The Tambre river is 125 km long and the surrounding basin covers an area of 1530 km^2, with an elongation ratio of 0.34. The Ulla river is 132 km long and drains an area of 2803 km^2, with an elongation ratio of 0.45 (Río Barja and Rodríguez Lestegás, 1992) (Fig. 1).

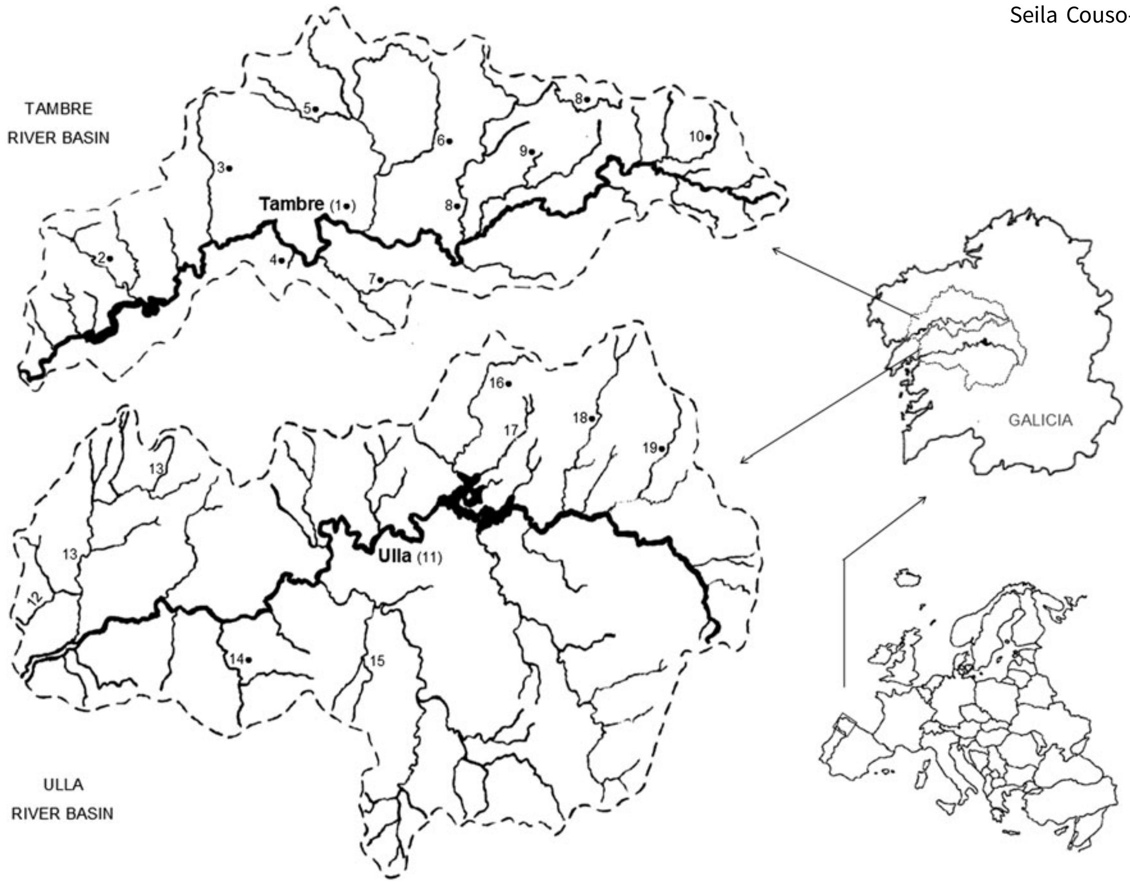

Fig. 1. Geographical location of the rivers in the Tambre and Ulla basins (Galicia, NW Spain) where brown trout specimens (*Salmo trutta*) were captured. (1) Tambre, (2) Barcala, (3) Dubra, (4) Oufín, (5) Paradela, (6) Lengüelle, (7) Sionlla, (8) Samo, (9) Gaiteiro, (10) Cabalar, (11) Ulla, (12) Rois, (13) Sar, (14) Liñares, (15) Toxa, (16) Iso, (17) Boente, (18) Furelos, (19) Pambre. •*Echinorhynchus truttae* presence.

During the 2015 fishing season (15 March–15 August), a total of 343 specimens of brown trout (*S. trutta*) were captured by local anglers, who removed the gastrointestinal tracts from the fish and stored them in hermetically sealed plastic bags at −20° C, before sending them to the Laboratory of Parasitology in the Faculty of Pharmacy (University of Santiago de Compostela) for analysis. The anglers also provided data such as the length of the fish and the river where they were caught. Fish age was estimated from length as described by Sánchez-Hernández *et al.* (2012) for specimens captured in the same study area: 19.0–19.1 cm (<2 years); 19.2–25.9 cm (2–3 years); >26.0 cm (>3 years).

The intestines were opened longitudinally and the adult forms of *E. truttae* were removed, washed with physiological saline solution and conserved in 70% ethanol. The parasites were stained with lactophenol cotton blue and identified following Crompton and Nickol (1985) and Buchmann and Bresciani (2001). The intestinal contents were also removed and ground in a mortar with 0.04 M phosphate-buffered saline (PBS) pH 7.2. The homogenates thus obtained were filtered through a set of two sieves (mesh size 150 and 45 µm) before being subjected to diphasic concentration in PBS (0.04 M pH 7.2)/diethyl ether (2:1) by centrifugation at 1250***g***, 4 °C, for 15 min. The supernatants were carefully discarded, and the concentration step was repeated until lipid-free sediments were obtained. Aliquots of 10 μL of the sediments were examined under bright field microscopy (×200 magnification) (AX70, Olympus Optical Co., Ltd., Tokyo, Japan). Prevalence rates, mean intensities and mean abundances of adults and eggs were used to describe the parasite infection according to Bush *et al.* (1997).

Statistical analyses were performed with Statgraphics® Centurion XVI v.16.2.04 Statistical Software (©1982–2013 StatPoint Technologies, Inc., Warrenton, VA, USA). Differences in the prevalence rates in relation to the river basin and fish age were investigated using Fisher's exact test and the χ^2 test. The mean intensities and mean abundances were compared by Mann–Whitney test. Differences were considered statistically significant at $P < 0.05$.

Results

Macroscopic and microscopic analysis of the intestinal contents of 343 brown trout revealed the presence of adults and/or eggs of *E. truttae* in 53 of the 123 trout from Tambre river basin (43.1%). By contrast, parasitic forms of this acanthocephalan parasite were only observed in eight of the 220 fish from the Ulla basin (3.6%), showing significant differences between the prevalences obtained in two adjacent watersheds ($P < 0.001$; odds ratio 19.0). The mean intensity and mean abundance of adult forms also differed significantly between the two river basins studied ($P < 0.01$) (Table 1). Furthermore, *E. truttae* was found in trout captured in all sampled rivers in the Tambre basin ($n = 10$), whereas the parasite was only detected in trout from four of the nine rivers sampled in the Ulla basin (Fig. 1).

Regarding the age of the specimens, both the prevalence and mean abundance of *E. truttae* adults in trout captured from Tambre river basin were significantly higher in fish older than 3 years that in younger trout (<2 and 2–3 years) ($P < 0.05$) (Table 1).

Discussion

Since the last quarter of the 20th century, when Cordero del Campillo and Álvarez Pellitero (1976) mentioned the presence of acanthocephalan species in brown trout (*S. trutta*) captured in Galician rivers, no data have been made available on these parasites in the geographical area considered in the present

https://doi.org/10.1017/pao.2017.24 Published online by Cambridge University Press

Table 1. Prevalence, mean intensity and mean abundance of *Echinorhynchus truttae* in brown trout (*Salmo trutta*) captured in two adjacent river basins in Galicia (NW Spain) in relation to the length and estimated age of the fish specimens

Watershed	Parameter	<2 years[a] (19.0[b]–19.1 cm)	2–3 years[a] (19.2–25.9 cm)	>3 years[a] (>26.0 cm)	Total
Tambre	*N*	48	62	13	123
	P	35.4	41.9	76.9	43.1
	MI adults	5.9 (1–16)	9.2 (1–45)	6.9 (1–25)	7.7 (1–45)
	MA adults ± SD	1.2 ± 3.3	2.5 ± 6.8	4.2 ± 7.1	2.2 ± 5.8
Ulla	*N*	65	134	21	220
	P	1.5	4.5	4.8	3.6
	MI adults	1.0 (1)	2.0 (1–4)	1.0 (1)	1.6 (1–4)
	MA adults ± SD	0.02 ± 0.0	0.04 ± 0.6	0.05 ± 0.0	0.04 ± 0.3

N, number of trout; *P*, prevalence (%); MI, mean intensity (adults/infected trout); MA, mean abundance (adults/examined trout); SD, standard deviation.
[a]Estimated according to the length of the fish following Sánchez-Hernández *et al.* 2012.
[b]Minimun legal size = 19.0 cm.

study. Álvarez Pellitero (1979) carried out a wide study on 1205 trout from several rivers in León, a region close to Galicia. However, this researcher did not detect any acanthocephalan parasites in the trout. To our knowledge, the present study is the first study providing data on the prevalence, mean intensity and mean abundance of *E. truttae* in brown trout in Spain.

The prevalences of infection by *E. truttae*, determined after macroscopic and microscopic examination of the intestines of 123 and 220 trout from Tambre and Ulla basins, were 43.1 and 3.6%, respectively. These values are within the range described for the same host in different areas of Europe. Thus, the prevalence of infection ranged between 5.5 and 93.3% for ten of 21 locations studied in Central Scotland (Dorucu *et al.* 1995), while the prevalence rates of 1.9, 17.4 and 47.1% were reported for trout captured in three streams in northern Italy (Dezfuli *et al.* 2001). Recently, in Turkey, Amin *et al.* (2016) reported a prevalence rate of 84.5% for *Echinorhynchus baeri* in *S. trutta*. By contrast, acanthocephalan species were not detected in 140 trout captured in four Irish lakes (Byrne *et al.* 2000) or in 484 fish captured in ten hydrographic drainage basins in the Mediterranean island of Corsica (Quilchini *et al.* 2010).

The effect of the fish size on both the abundance and richness of helminth parasites is well documented (Guégan and Hugueny, 1994). Higher prevalence and abundance in older trout can be attributed to a higher infection risk than younger specimens because older fish are exposed to infective prey for a longer time period and, also, larger fish ingest greater amounts of prey. Dezfuli *et al.* (2001) observed that the number of *E. truttae* adults per fish was significantly correlated with fish length in one of the three streams studied in northern Italy. Similarly, in our study, the percentage of positive samples and the number of *E. truttae* adults per fish were higher in trout of length >26 cm than in smaller specimens. This correlation was observed in trout captured in the Tambre basin, but it was not detected in specimens from the Ulla basin, possibly due to the small number of positive samples obtained from this river basin.

The diet of trout is mainly determined by habitat, season, prey availability and ontogeny (Knutsen *et al.* 2001; Lehane *et al.* 2001; Lagarrigue *et al.* 2002). As the fish grow, their diet changes both qualitatively and quantitatively and they feed on larger macroinvertebrates as well as on other more energetically valuable prey (Oscoz *et al.* 2000) and, therefore, having higher risk of acquiring parasitic infection through the trophic chain. Prey selection by trout can also play a role in the pattern of *E. truttae* infection. However, Sánchez Hernández (2009) concluded, after the study of stomach contents of brown trout captured from rivers in the Tambre and Ulla basins, that the feeding of the trout of this geographical area is determined by the most abundant and widely distributed prey through the river.

The parasitization of *S. trutta* by the acanthocephalan parasite *E. truttae* implies ingestion of gammarid amphipods harbouring the cystacanth form. This infective form can modify the crustacean behaviour so that it reacts differently to light, becoming more positively phototropic, active and swimming closer to the water surface. The crustaceans thus become more conspicuous to fish and more vulnerable to predation by the definitive host (Fielding *et al.* 2003; MacNeil *et al.* 2003; Lagrue *et al.* 2013). Unfortunately, we do not have any data on the parasite status of the gammarid amphipod. However, the different prevalence rates of *E. truttae* suggest that the presence/abundance of the crustacean that acts as the intermediate host may be different in the two river basins. This is supported by information provided by the Environmental Observation Network of Environmental Laboratory (ROAGA-LMAG, Xunta de Galicia) (http://siam.xunta.gal/roaga-lma-descricion) on benthic macroinvertebrate fauna inhabiting several water bodies in the Tambre and Ulla river basins, indicating a higher abundance of gammarids in the Tambre basin (data not shown). Moreover, differences in the prevalence rates of helminth parasites in fish from close water bodies were observed by other authors. Hartvigsen and Kennedy (1993) carried out studies on composition and richness of helminth communities in *S. trutta* from ten water reservoirs situated close to each other in a well-defined region of south-west England, and observed that local factors promoting distinctiveness have a greater influence than regional factors which induce similarity.

According to previous reports, the amphipods involved in the biological cycle of *E. truttae* are species of the genus *Gammarus*, specifically *Gammarus pulex* (Crompton and Nickol, 1985; Kennedy, 2006). Although we did not find any reference to the presence of this species in freshwater environments in the Iberian Peninsula, at least ten other species of the genus *Gammarus* have been described (García and Jaume, 2008). The Gammaridae family is the most common group of crustaceans in freshwater environments in Galicia, with *Echinogammarus lusitanicus* being the most abundant species, although *Echinogammarus beriyoni* is also present in the middle and low stretches of the rivers (González González and Cobo Gradín, 2006). As occurs in Ireland with the indigenous *Gammarus duebeni* (MacNeil *et al.* 2000; Prenter *et al.* 2004), our findings suggest that native Galician gammarid species may act as intermediate hosts of *E. truttae*, although this remains to be confirmed.

Finally, the remarkable difference in the prevalence of *E. truttae* in brown trout captured in Tambre and Ulla basins may be a consequence of various conditions such as lack of oxygen due to organic pollution and low pH (Meijering, 1991), temperature and salinity changes (Foucreau *et al.* 2014; Vereshchagina *et al.* 2016), recognized as important factors which influence and alter natural distribution of gammarid amphipods, intermediate hosts for this acanthocephalan parasite.

Acknowledgements. The authors thank anonymous local anglers for their collaboration and the Environmental Observation Network of Environmental Laboratory (ROAGA-LMAG, Xunta de Galicia) for providing information about benthic macroinvertebrate communities.

Financial support. The study was funded by the Autonomous Government of Galicia (grants GPC2014/069 and ED431C 2017/31).

Conflict of interest. None.

References

Álvarez Pellitero MP (1979). *Helmintosis de la trucha en León*. PhD Thesis, University of León, León, Spain.

Amin OM, Heckmann RA, Evans RP and Tepe Y (2016) A description of *Echinorhynchus baeri* Kostylew, 1928 (Acanthocephala: Echinorhynchidae) from *Salmo trutta* in Turkey, with notes on synonymy, geographical origins, geological history, molecular profile, and X-ray microanalysis. *Parasite* **23**, 56. doi: 10.1051/parasite/2016067.

Buchmann K and Bresciani J (2001) *An Introduction to Parasitic Diseases of Freshwater Trout*, 1st edn. Frederiksberg, Denmark: DSR Publishers.

Bush AO, Lafferty KD, Lotz JM and Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis *et al.* Revisited. *Journal of Parasitology* **83**, 575–583.

Byrne CJ, Grey C, Holland C and Poole R (2000) Parasite community similarity between four Irish lakes. *Journal of Helminthology* **74**, 301–305. doi: https://doi.org/10.1017/S0022149X00000445.

Cordero del Campillo M and Álvarez Pellitero MP (1976) *Los Parásitos de las Truchas en España*. In Grupo Sindical de Piscicultores (ed.). *Tratado de Piscicultura. I Jornadas Nacionales de la Trucha*. León, Spain: SINA, pp. 507–523.

Crompton DWT and Nickol BB (1985) *Biology of the Acanthocephala*, 1st edn. Cambridge University Press, Cambridge, United Kingdom.

Dezfuli BS, Giari L, Biaggi DS and Poulin R (2001) Associations and interactions among intestinal helminths of the brown trout, *Salmo trutta*, in northern Italy. *Journal of Helminthology* **75**, 331–336. doi: 10.1017/S0022149X01000518.

Dorucu M, Crompton DWT, Huntingford FA and Walters DE (1995) The ecology of endoparasitic helminth infections of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) in Scotland. *Folia Parasitologica* **42**, 29–35.

Fielding NJ, MacNeil C, Dick JTA, Elwood RW, Riddell G and Dunnam AM (2003) Effects of the acanthocephalan parasite *Echinorhynchus truttae* on the feeding ecology of *Gammarus pulex* (Crustacea: Amphipoda). *Journal of Zoology, London* **261**, 321–325. doi: 10.1017/S0952836903004230.

Foucreau N, Cottin D, Piscart C and Hervant F (2014) Physiological and metabolic responses to rising temperature in *Gammarus pulex* (Crustacea) populations living under continental or Mediterranean climates. *Comparative Biochemistry and Physiology, Part A* **168**, 69–75. doi: 10.1016/j.cbpa.2013.11.006.

García L and Jaume D (2008) Fauna ibérica. Museo Nacional de Ciencias Naturales (MNCN) – Centro Superior de Investigaciones Científicas (CSIC), Ministerio de Economía, Industria y Competitividad (MINECO) Spain. In: Available at http://www.faunaiberica.mncn.csic.es/faunaib/arthropoda/crustacea/amphipoda.php. Accessed 27 November 2017.

Gilbert BM and Avenant-Oldewage A (2017) Parasites and pollution: the effectiveness of tiny organisms in assessing the quality of aquatic ecosystems, with a focus on Africa. *Environmental Science and Pollution Research International* **24**, 18742–18769. doi: 10.1007/s11356-017-9481-8.

González González MA and Cobo Gradín F (2006) *Macroinvertebrados de las Aguas Dulces de Galicia*. Hércules de Ediciones, A Coruña, Spain.

Guégan JF and Hugueny B (1994) A nested parasite species subset pattern in tropical fish: host as major determinant of parasite infracommunity structure. *Oecologia* **100**, 184–189. doi: 10.1007/BF00317145.

Hartvigsen R and Kennedy CR (1993) Patterns in the composition and richness of helminth communities in brown trout, *Salmo trutta*, in a group of reservoirs. *Journal of Fish Biology* **43**, 603–615. doi: 10.1111/j.1095-8649.1993.tb00443.x.

Kennedy CR (2006) *Ecology of the Acanthocephala*. 1st edn. Cambridge University Press, Cambridge, UK.

Knutsen JA, Knutsen H, Gjosaeter J and Jonsson B (2001) Food of anadromous brown trout at sea. *Journal of Fish Biology* **59**, 553–543. doi: 10.1111/j.1095-8649.2001.tb02359.x.

Lagarrigue T, Céréghino R, Lim P, Reyes-Marchánt P., Chappaz R., Lavandier P. and Belaud A. (2002) Diel and seasonal variations in brown trout (*Salmo trutta*) feeding patterns and relationship with invertebrate drift under natural and hydropeaking conditions in a mountain stream. *Aquatic Living Resources* **15**, 129–137. doi: 10.1016/S0990-7440(02)01152-X.

Lagrue C, Güvenatam A and Bollache L (2013) Manipulative parasites may not alter intermediate host distribution but still enhance their transmission: field evidence for increased vulnerability to definitive host and non-host predator avoidance. *Parasitology* **140**, 258–265. doi: 10.1017/S0031182012001552.

Lehane BM, Walsh B, Giller PS and O'Halloran J (2001) The influence of small-scale variation in habitat on winter trout distribution and diet in an afforested catchment. *Aquatic Ecolology* **61**, 61–71. doi: 10.1023/A:1011467711628.

MacNeil C, Dick JTA and Elwood RW (2000) Differential physico-chemical tolerances of amphipod species revealed by field transplantations. *Oecologia* **124**, 1–7. doi: https://doi.org/10.1007/PL00008864.

MacNeil C, Fielding NJ, Hume KD, Dick JTA, Elwood RW, Hatcher MJ and Dunnam AM (2003) Parasite altered micro-distribution of *Gammarus pulex* (Crustacea: Amphipoda). *International Journal for Parasitology* **33**, 57–64. doi: 10.1016/S0020-7519(02)00229-1.

Meijering MPD (1991) Lack of oxygen and low pH as limiting factors for *Gammarus* in Hessian brooks and rivers. *Hydrobiologia* **223**, 159–169. doi: https://doi.org/10.1007/BF00047637.

Oscoz J, Escala MC and Campos F (2000) La alimentación de la trucha común (*Salmo trutta* L., 1758) en un río de Navarra (N. España). *Limnetica* **18**, 29–35.

Prenter J, MacNeil C, Dick JTA, Riddell GE and Dunn AM (2004) Lethal and sublethal toxicity of ammonia to native, invasive, and parasitized freshwater amphipods. *Water Research* **38**, 2847–2850. doi: 10.1016/j.watres.2004.03.042.

Quilchini Y, Foata J, Mouillot D, Mattei J and Marchand B (2010) The influence of altitude, hydrographic network and season on brown trout parasites in Corsica using indicator species analysis. *Journal of Helminthology* **84**, 13–19. doi: 10.1017/S0022149X09990101.

Río Barja FJ and Rodríguez Lestegás F (1992) *Os Ríos Galegos. Morfoloxía e réxime*. Consello da Cultura Galega, Santiago de Compostela, Spain.

Sánchez Hernández J (2009) *Biología de la alimentación de la trucha común (Salmo trutta Linné, 1758) en los ríos de Galicia*. PhD Thesis, University of Santiago de Compostela, Santiago de Compostela, Spain.

Sánchez-Hernández J, Servia MJ, Vieira-Lanero R, Barca-Bravo S and Cobo F (2012) Reference data on the growth and population parameters of brown trout in siliceous rivers of Galicia (NW Spain). *Limnetica* **31**, 267–282.

Sures B (2004) Environmental parasitology: relevancy of parasites in monitoring environmental pollution. *Trends in Parasitology* **20**, 170–177. doi: 10.1016/j.pt.2004.01.014.

Vereshchagina KP, Lubyaga YA, Shatilina Z, Bedulina D, Gurkov A, Axenov-Gribanov DV, Baduev B, Kondrateva ES, Gubanov M, Zadereev E, Sokolova I and Timofeyev M (2016) Salinity modulates thermotolerance, energy metabolism and stress response in amphipods *Gammarus lacustris*. *PeerJ* **4**, e2657. Doi: 10.7717/peerj.2657.

Wayland MT (2013) Morphological variation in *Echinorhynchus truttae* Schrank, 1788 and the *E. bothniensis* Zdzitowiecki & Valtonen, 1987 species complex from freshwater fishes of Northern Europe. *Biodiversity Data Journal* **1**, e975. doi: 10.3897/BDJ.1.e975.