

Proceedings



Cryptosporidium Species in Fish: An Update +

Golomazou E.¹ and Karanis P.^{2,3,*}

- Department of Ichthyology and Aquatic Environment-Aquaculture Laboratory, School of Agricultural Sciences, University of Thessaly, 38446 Volos, Greece; egolom@uth.gr
- ² Medical Faculty and University Hospital, University of Cologne, 50931 Cologne, Germany
- ³ Department of Basic and Clinical Sciences, Anatomy Centre, University of Nicosia Medical School, 2408 Nicosia, Cyprus
- * Correspondence: karanis.p@unic.ac.cy; Tel.: +357-22-471813
- + Presented at the 4th EWaS International Conference: Valuing the Water, Carbon, Ecological Footprints of Human Activities, Online, 24–27 June 2020.

Published: 11 August 2020

Abstract: *Cryptosporidium* species have been detected in fish, in a wide range of geographical regions. Currently, three species are genetically characterized as piscine-host-specific: *C. molnari, C. scophthalmi, C. huwi*. One potential novel species, 23 additional piscine genotypes, other non-piscine-host-specific *Cryptosporidium* species (*C. parvum, C. hominis, C. scrofarum, C. xiaoi*) and the rat genotype III have been genetically characterized in fish. The pathology of cryptosporidiosis is very important for the aquaculture industry, causing mortalities in farmed fish, while the presence of *Cryptosporidium* zoonotic subtypes in edible fish increases the fish-borne zoonotic potential risk, which is of major importance from a public health point of view.

Keywords: Cryptosporidium spp.; fish; aquaculture; zoonosis

1. Introduction

Cryptosporidium spp. are included in the "top ten" list of foodborne parasites of greatest global concern, which affect people's health, infecting tissues and causing clinical symptoms [1]. *Cryptosporidium* is identified as the second most common cause of diarrhoea worldwide and death in children, after rotavirus [2]. Symptoms of cryptosporidiosis include diarrhoea, vomiting, nausea, lack of appetite and cramps. There is no effective treatment or vaccine available. It is usually a self-limiting disease in the case of immunocompetent individuals but can be chronic in immunocompromised people and young children. It is caused by the enteric protozoan parasite of the genus *Cryptosporidium*, which infects a huge range of domestic animals, wildlife population and humans [3].

The current knowledge on *Cryptosporidium* species infecting piscine hosts is steadily increasing, since *Cryptosporidium* species have been detected in marine and freshwater, cultured, captive and ornamental fish, in a wide range of geographical regions. The presence of zoonotic *Cryptosporidium* subtypes in fish samples has been described in various studies [4–7]. Fish-borne zoonotic potential risk from *Cryptosporidium* species is of major importance from a public health point of view. The presence of these parasites in edible fish may be the etiological agent responsible for outbreaks of fish-borne cryptosporidiosis. It may also produce a variety of clinical signs in infected fish, which are more obvious in the case of heavy infections. The pathology of cryptosporidiosis in cultured fish is important, followed by poor growth rates and increased mortality [8,9] causing an economic impact in the aquaculture industry, which is expanding worldwide.

2. Cryptosporidium Species in Piscine Hosts

Cryptosporidium species have been detected in marine and freshwater, cultured, captive and ornamental fish in a wide range of geographical regions (Figure 1). Most commonly, the parasite has been reported in Australia, where 58% of worldwide reports have been recorded. The first piscine host of *Cryptosporidium* was the tropical fish *Naso lituratus*, in which *C. nasorum* was identified [10]. To date, three species are genetically characterized as piscine-host-specific: *C. molnari*, *C. scophthalmi*, *C. huwi*, which are the main three recorded piscine species (Table 1). *C. molnari* has been genetically characterized in gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) [11,12]. *C. huwi* has been identified in the ornamental guppy (*Poecilia reticulata*) [13], the golden tiger barb (*Puntigrus tetrazona*) [14] and the Neon tetra (*Paracheirodon innesi*) [15]. *C. scophthalmi* was firstly reported in turbot [8], but no molecular data are available. However, molecular characterization has identified a *C. scophthalmi*-like organism in *Scophthalmus maximus* [16]. These main three recorded piscine species have been reported mainly in Spain, France, Australia and North East Atlantic, while *C. molnari* and similar genotypes is the species with the widest distribution (Figure 2). One potential novel species has been isolated in angelfish [17] in Washington State, USA.



Figure 1. Geographical distribution of *Cryptosporidium* reports on piscine hosts, recorded worldwide.



C. molnari C. molnari-like C. scophthalmi C. huwi

Figure 2. Geographical distribution of piscine host specific Cryptosporidium species' reports.

Parasite Species	Host	Origin	Geog. Origin	References
C. nasorum	Naso tang Naso lituratus	M/O	Indiana USA	[10]
C. molnari	Gilthead sea bream Sparus aurata	M/C	Spain	[9,11,24]
	European sea bass Dicentrarchus labrax	M/C	Spain	[9,11,24]
	Murray cod Maccullochella peelii	F/C	Australia	[25]
	Northern pike Esox lucius	F/W	France	[6]
	Bristle tooth tang Ctenochaetus tominiensis	M/O	Australia	[18]
	Butter bream Monodactylus argenteus	M/O	Australia	[18]
	Madder seaperch Pseudodanthias dispar	M/O	Australia	[18]
	Golden algae eater Crossocheilus aymonieri	F/O	Australia	[18]
	Green chromis Chromis viridis	M/O	Australia	[18]
	Upside down cat fish Synodontis nigriventris	F/O	Australia	[18]
	Wedgetailed blue tang Paracanthurus hepatus	M/O	Australia	[18]
C. molnari-like	Angelfish Pterophyllum altum	F/O	Australia	[15]
	Azure damsel Chrysiptera hemicyanea	M/O	Australia	[14,15]
	Goldfish Carassius auratus	F/O	Australia	[14,15]
	Guppy Poecilia reticulata	F/O	Australia	[15]
	Orange clownfish Amphiprion percula	M/O	Australia	[15]
	Oscar Astronotus ocellatus	F/O	Australia	[15]
	Peach anthias Pseudanthias dispar	M/O	Australia	[14,15]
	Red-striped angelfish Centropyge eibli	M/O	Australia	[14,15]
	Yellow-headed jawfish Opistognathus aurifrons	M/O	Australia	[14,15]
	Cod Gadus morhua	M/W	NEA	[21]
	Brown trout Salmo trutta	F/W	Spain	[26]
C. scophthalmi	Turbot Scophthalmus maximus	M/C	Spain	[8,16]
	Turbot Psetta maxima	M/C	Spain	[27]
C. huwi	Guppy Poecilia reticulata	F/O	Australia	[13–15]
	Golden tiger barb Puntigrus tetrazona	F/O	Australia	[13–15]
	Neon tetra Paracheirodon innesi	F/O	Australia	[13–15,18]

Table 1. Piscine (Cryptosporidium	species.
--------------------	-----------------	----------

Origin: Marine (M), Freshwater (F), Cultured (C), Wild (W), Ornamental (O), Laboratory fish (L). Geog. Origin: Geographic origin, NEA:North East Atlantic, EEC: Eastern English channel, NS: Norwegian sea.

According to molecular data, piscine genotypes 2–8 (PG2–PG8) [5,13,14,19], genotype 9 (G9) [7] and novel genotypes (NG, NGC1–5,7), one novel genotype similar to *C. molnari* in koi carp causing disseminated infection [20] and 14 un-named novel genotypes [4,14,15,21] are additional piscine genotypes (Table 2), which have been reported worldwide, as described in Figure 3.

Non-piscine-host-specific *Cryptosporidium* species such as *C. parvum*, *C. hominis*, *C. scrofarum*, *C. xiaoi* and the rat genotype III have been identified in fish [3–7,19,21–23], indicating the contamination of the aquatic environment from the agricultural runoff and discharged sewage.

Genotypes	Host	Origin	Geog. Origin	References
PG 2	Neon tetra Paracheirodon innesi	F/O		[18]
	Oscar Astronotus ocellatus	F/O	Australia	[13–15,18]
	Mullet Mugil cephalus	M/W		[15]
PG 3	Mullet Mugil cephalus	M/W	A	[15]
PG 3-like	Goldfish Carassius auratus	F/O	Australia	[15]
PG 4	Golden algae eater Crossocheilus aymonieri	F/O	Australia	[18]
	Kupang damsel Chrysiptera hemicyanea	M/O		[18]
	Oscar Astronotus ocellatus	F/O		[18]
	Neon tetra Paracheirodon innesi	F/O		[19]
	Azure damsel Chrysiptera hemicyanea	F/O		[15]
	Black ghost knife fish Apteronotus albifrons	F/O		[15]
	Kribensis Pelvicachromis pulcher	F/O		[15]
	Angel fish Pterophyllum scalare Pterophyllum altum	F/O	Australia	[15,18]
	Butter bream Monodactylus argenteus	M/O		[15,18]
PG 5	Golden algae eater Crossocheilus aumonieri	F/O		[18]
	Black ghost knife fish Apteronotus albifrons	F/O		[15]
	Blue tang <i>Paracanthurus hepatus</i>	M/O		[14,15]
	Goldfish Carassius auratus	F/O		[15]
	Guppy Poecilia reticulata	F/O		[15]
	Mullet Mugil cephalus	M/W		[15]
	Platyfish Xiphophorus maculatus	F/O		[15]
PG 6	Guppy Poecilia reticulata	F/O	Australia	[18]
PG 6-like	Gold gourami Trichogaster trichopterus	F/O		[19]
PG 7	Red eye tetra Moenkhausia sanctaefilomenae	F/O	Australia	[19]
	Neon tetra Paracheirodon innesi	F/O		[15]
PG 7-like	Neon tetra Paracheirodon innesi	F/O		[15]
PG8	Oblong silver biddy Gerres oblongus	M/W,M/O	Australia	[5,15]
G9	Rainbow trout Oncorhynchus mykiss	F/C	Spain	[7]
NG	Azure damsel Chrysiptera hemicyanea	F/O	Australia	[14,15]
	Sea mullet Mugil cephalus	M/W		[4]
	Orange clownfish Amphiprion percula	M/O		[14,15]
	Oscar Astronotus ocellatus	F/O		[15]
	Platyfish Xiphophorus maculatus	F/O		[15]
	Koi carp Cyprinus carpio	F/O	Netherlands	[20]
NICC1	Saithe Pollachius virens	M/W	NEA, EEC, NS	[21]
	Blue ling Molva dypterygia	M/W	NEA	[21]
NGC2	Whiting Merlangius merlangus	M/W	NEA	[21]

	Ling Molva molva	M/W	NEA	[21]
NGC3	Ling Molva molva	M/W	NEA	[21]
NGC4	Blue ling Molva dypterygia	M/W	NEA	[21]
NGC5	Saithe Pollachius virens	M/W	NEA, EEC, NS	[21]
	Hake Merluccius merluccius	M/W	NEA	[21]
NGC7	Mackerel Scomber scombrus	M/W	EEC	[21]

Origin: Marine (M), Freshwater (F), Cultured (C), Wild (W), Ornamental (O), Laboratory fish (L). Geog. Origin: Geographic origin, NEA:North East Atlantic, EEC: Eastern English channel, NS: Norwegian sea.



Figure 3. Worldwide distribution of piscine genotypes' reports.

3. Cryptosporidium Zoonotic Species in Fish

Humans are susceptible to a wide range of *Cryptosporidium* spp. and genotypes, with *C. hominis* and *C. parvum* as the main species infecting humans globally [22–24]. According to a recent evolutionary genomic survey of anthroponotic *Cryptosporidium* species, *C. hominis* and *C. p. anthroponosum* are the main parasites that primarily infect humans, as *C. parvum* can be further divided into two branches with distinct host associations: the *C. parvum anthroponosum* (anthroponotic) and the *C. parvum parvum* (zoonotic) [25].

Several *Cryptosporidium* species have been detected in fish. The presence of zoonotic subtypes in fish is probably related to water contamination by animal and human wastes. *C. parvum* zoonotic subtypes are of major significance to public health, as they represent the most common source of zoonotic infection [3]. This has been described in various studies [4–7] including a great range of hosts and geographic regions. During the last decade, it has been detected in 18 freshwater and marine fish species including edible fish, both wild and farmed. Prevalence of infection was low in most cases. Trout species are recorded as the most sensitive piscine host, as the highest prevalence has been recorded in case of Brown trout [26] and European trout in NW Spain [7].

C. parvum developmental stages are mainly detected in the digestive track (stomach, pyloric caeca, intestine). However, the isolated presence of other non-pathogenic *Cryptosporidium* species in fish fillets clearly highlights the risk of infection to humans [6]. This fact indicates the potential zoonotic risk of transmission from the fish digestive tract to the fish fillet, in case of *C. parvum* infected edible fish. *Cryptosporidium* spp. can be transmitted either by consumption of undercooked fish, or

by contact with fish during preparation and handling, especially in immunosuppressed patients [27,28].

4. Pathology

Cryptosporidium is an obligate intracellular protozoan parasite that mainly infects the microvillus border of the gastrointestinal epithelium. In contrast with the epicellular location of *Cryptosporidium* species from other vertebrates, in case of piscine *Cryptosporidium* species sporulation takes place deep within the epithelium [11–13].

The pathology of cryptosporidiosis is related to host sensitivity and its response against the parasite. In some cases, the prevalence and the intensity of infection is low and the pathological impact is absent [6,29] or very mild, while oocysts appear degenerated and surrounded by host cellular reaction, as in the case of seabass infected with *C. molnari* [11].

When severe parasitosis occurs, infected fish are in poor physical condition, characterized by listlessness, muscle wasting and atrophy. Poor growth rates and increased mortality are recorded, while anorexia, severe emaciation or swollen coelomic cavities and abdominal swelling ascites have been observed. During necropsy, indigestion, intestinal distension arising from mucous intestinal contents, gas and whitish faeces are the main findings [8,10,17,18].

Piscine cryptosporidiosis may produce a variety of clinical signs in infected fish, which are more obvious in case of heavy infections. The histopathological effect of the parasite is similar in most cases, as necrosis, vacuolisation, degradation, disaggregation and sloughing of epithelial cells is observed in infected tissues [30]. In the case of gastric cryptosporidiosis, organisms are distributed among the gastric mucosa. Gastritis is present in infected fish, varying from mild to moderate, depending on the intensity of the parasitosis [31]. When inflammation is severe, necrosis of gastric mucosal epithelial cells, mucosal glandular atrophy and glandular loss may be present. Vacuolation appears surrounding oocysts engulfed by hypertrophied cells, probably macrophages. Massive vacuolation and abundant debris appear within the cells and in the lumen in treated fish, while in severely affected fish, clusters of oocysts occupy wide vacuolated areas of the intestinal epithelium [31]. Infected intestines also show different degrees of damage, usually correlated with the infection intensity. Displacement of the microvilli and focal indentation at sites of attachment of the parasites on the mucosal epithelium is recorded [10]. In moderate infections, mild intestinal lesions are observed, while in massive infections severe intestinal lesions are mentioned and most of the epithelial surface is covered by oocysts. In such advanced infections, normal mucosal architecture is distorted, leading to desquamation of the intestinal mucosa, and epithelial cells are distended by large vacuoles containing clusters of oocysts. In these cases, the inflammatory response may be severe and exocytosis of some lymphocytes into the epithelium is common. The abundance of rodlet cells in infected epithelia is also evident and numerous goblet cells are also observed [8].

Cryptosporidium spp. sporogonial stages, apart from gastrointestinal track, have been detected in other tissues. The parasite has been recorded in bile duct epithelial cells of turbot, causing the fibrosis of the bile ducts and necrosis of adjacent hepatic cells [32]. The liver was also a target organ in the case of Koi carp, being pale brown with severe granulomatous inflammatory lesions [20]. In this case, superficial haemorrhages in the skin and hyperaemia of the gills were present, while the spleen was swollen and irregularly granular. Extensive inflammation was evident on histological analysis, with many granulomas present in the kidneys, the spleen, the base of the secondary lamellae and the cartilage of the gills and between the skeletal muscles. The intestines were empty and pale with a thickened mucosa and haemorrhages, while many granulomas were present in the tip of the villi in the lamina propria and near the crypts [20].

5. Conclusions

Cryptosporidium species have been recorded in a wide range of piscine hosts worldwide. The presence of *Cryptosporidium* zoonotic subtypes in edible fish, which are commonly consumed raw or slightly processed, increases the fish-borne zoonotic potential risk. Furthermore, the pathology of cryptosporidiosis in fish is very important. Intensive aquaculture practices, stressful conditions, high

host density, water supply and temperature, in combination with the immunological immaturity of the host, increase the prevalence, the mortality rate and interactions with other pathogens in farmed fish, having a significant economic impact on the aquaculture industry.

Author Contributions: All authors have read and agree to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. FAO Identifies Top 10 Foodborne Parasites. Vet. Rec. 2014, 175, 58.
- 2. Kotloff, K.L.; Nataro, J.P.; Blackwelder, W.C.; Nasrin, D.; Farag, T.H.; Panchalingam, S. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): A prospective, case-control study. *Lancet* **2013**, *382*, 209–222.
- 3. Ryan, U.; Fayer, R.; Xiao, L. *Cryptosporidium* species in humans and animals: Current understanding and research needs. *Parasitology* **2014**, *141*, 1667–1685.
- 4. Reid, A.; Lymbery, A.; Ng, J.; Tweedle, S.; Ryan, U. Identification of novel and zoonotic *Cryptosporidium* species in marine fish. *Vet. Parasitol.* **2010**, *168*, 190–195.
- 5. Koinari, M.; Karl, S.; Ng-Hublin, J.; Lymbery, A.J.; Ryan, U.M. Identification of novel and zoonotic *Cryptosporidium* species in fish from Papua New Guinea. *Vet. Parasitol.* **2013**, *198*, 1–9.
- Certad, G.; Dupouy-Camet, J.; Gantois, N.; Hammouma-Ghelboun, O.; Pottier, M.; Guyot, K.; Benamrouz, S.; Osman, M.; Delaire, B.; Creusy, C.; et al. Identification of *Cryptosporidium* species in fish from lake Geneva (Lac Léman) in France. *PLoS ONE* 2015, *10*, e0133047.
- Couso-Pérez, S.; Ares-Mazás, E.; Gómez-Couso, H. Identification of a novel piscine *Cryptosporidium* genotype and *Cryptosporidium parvum* in cultured rainbow trout (*Oncorhynchus mykiss*). *Parasitol. Res.* 2018, 117, 2987–2996.
- Alvarez-Pellitero, P.; Quiroga, M.I.; Sitjà-Bobadilla, A.; Redondo, M.J.; Palenzuela, O.; Padrós, F.; Vázquez, S.; Nieto, J.M. *Cryptosporidium scophthalmi* n. sp. (Apicomplexa: Cryptosporidiidae) from cultured turbot *Scophthalmus maximus*. Light and electron microscope description and histopathological study. *Dis. Aquat. Organ.* 2004, *62*, 133–145.
- 9. Sitjà-Bobadilla, A.; Padrós, F.; Aguilera, C.; Alvarez-Pellitero, P. Epidemiology of *Cryptosporidium molnari* in Spanish gilthead sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.) cultures: From hatchery to market size. *Appl. Environ. Microbiol.* **2005**, *71*, 131–139.
- 10. Hoover, D.M.; Hoerr, F.J.; Carlton, W.W.; Hinsman, E.J.; Ferguson, H.W. Enteric cryptosporidiosis in a naso tang, *Naso lituratus* Bloch and Schneider. *J. Fish Dis.* **1981**, *4*, 425–428.
- 11. Alvarez-Pellitero, P.; Sitjà-Bobadilla, A. *Cryptosporidium molnari* n. sp. (Apicomplexa: Cryptosporidiidae) infecting two marine fish species, *Sparus aurata* L. and *Dicentrarchus labrax* L. *Int. J. Parasitol.* **2002**, *32*, 1007–1021.
- 12. Palenzuela, O.; Alvarez-Pellitero, P.; Sitjá-Bobadilla, A. Molecular characterization of *Cryptosporidium molnari* reveals a distinct piscine clade. *Appl. Environ. Microbiol.* **2010**, *76*, 7646–7649.
- Ryan, U.; Paparini, A.; Tong, K.; Yang, R.; Gibson-Kueh, S.; O'Hara, A.; Lymbery, A.; Xiao, L. *Cryptosporidium huwi* n. sp. (Apicomplexa: Eimeriidae) from the guppy (*Poecilia reticulata*). *Exp. Parasitol.* 2015, 150, 31–35.
- Paparini, A.; Yang, R.; Chen, L.; Tong, K.; Gibson-Kueh, S.; Lymbery, A.; Ryan, U.M. *Cryptosporidium* in fish: Alternative sequencing approaches and analyses at multiple loci to resolve mixed infections. *Parasitol.* 2017, 144, 1811–1820.
- Yang, R.; Palermo, C.; Chen, L.; Edwards, A.; Paparini, A.; Tong, K.; Gibson-Kueh, S.; Lymbery, A.; Ryan, U. Genetic diversity of *Cryptosporidium* in fish at the 18S and actin loci and high levels of mixed infections. *Vet. Parasitol.* 2015, *214*, 255–263.
- 16. Costa, J.F.; Saraiva, A. *Cryptosporidium* cf. *scophthalmi* JFC-2015 18S Ribosomal RNA Gene, Partial Sequence. 2015. Available online: https://www.ncbi.nlm.nih.gov/nuccore/KR340588.1 (accessed on 15 May 2020).
- 17. Murphy, B.G.; Bradway, D.; Walsh, T.; Sanders, G.E.; Snekvik, K. Gastric cryptosporidiosis in freshwater angelfish (*Pterophyllum scalare*). J. Vet. Diagn. Investig. 2009, 21, 722–727.
- 18. Zanguee, N.; Lymbery, J.A.; Lau, J.; Suzuki, A.; Yang, R.; Ng, J.; Ryan, U. Identification of novel *Cryptosporidium* species in aquarium fish. *Vet. Parasitol.* **2010**, *174*, 43–48.

- 19. Morine, M.; Yang, R.; Ng, J.; Kueh, S.; Lymbery, A.J.; Ryan, U.M. Additional novel *Cryptosporidium* genotypes in ornamental fishes. *Vet. Parasitol.* **2012**, *190*, 578–582.
- 20. Yang, R.; Dorrestein, G.M.; Ryan, U. Molecular characterisation of a disseminated *Cryptosporidium* infection in a Koi carp (*Cyprinus carpio*). *Vet. Parasitol.* **2016**, *226*, 53–56.
- Certad, G.; Follet, J.; Gantois, N.; Hammouma-Ghelboun, O.; Guyot, K.; Benamrouz-Vanneste, S.; Fréalle, E.; Seesao, Y.; Delaire, B.; Creusy, C.; et al. Prevalence, molecular identification, and risk factors for *Cryptosporidium* infection in edible marine fish: A survey across sea areas surrounding France. *Front. Microbiol.* 2019, 10, 1037.
- 22. Xiao, L. Molecular epidemiology of cryptosporidiosis: An update. Exp. Parasitol. 2010, 124, 80-89.
- 23. Ryan, U.; Zahedi, A.; Paparini, A. *Cryptosporidium* in humans and animals—A one health approach to prophylaxis. *Parasite Immunol.* **2016**, *38*, 535–547.
- 24. Efstratiou, A.; Ongerth, J.E.; Karanis, P. Waterborne transmission of protozoan parasites: Review of worldwide outbreaks—An update 2011–2016. *Water Res.* **2017**, *114*, 14–22.
- Nader, J.L.; Mathers, T.C.; Ward, B.J.; Pachebat, J.A.; Swain, M.T.; Robinson, G.; Chalmers, R.M.; Hunter, P.R.; van Oosterhout, C.; Tyler, K.M. Evolutionary genomics of anthroponosis in *Cryptosporidium*. *Nat. Microbiol.* 2019, *4*, 826–836.
- 26. Couso-Pérez, S.; Ares-Mazás, E.; Gómez-Couso, H. First report of *Cryptosporidium molnari*-like genotype and *Cryptosporidium parvum* zoonotic subtypes (IIaA15G2R1 and IIaA18G3R1) in brown trout (*Salmo trutta*). *J. Parasitol.* **2019**, *105*, 170.
- 27. McOliver, C.C.; Lemerman, H.B.; Silbergeld, E.K.; Moore, R.D.; Graczyk, T.K. Risks of recreational exposure to waterborne pathogens among persons with HIV/AIDS in Baltimore, Maryland. *Am. J. Public Health* **2009**, *99*, 1116–1122.
- 28. Graczyk, T.K.; McOliver, C.; Silbergeld, E.K.; Tamang, L.; Roberts, J.D. Risk of handling as a route of exposure to infectious waterborne *Cryptosporidium parvum* oocysts via Atlantic blue crabs (*Callinectes sapidus*). *Appl. Environ. Microbiol.* **2007**, *73*, 4069–4070.
- 29. Alvarez-Pellitero, P.; Perez, A.; Quiroga, M.I.; Redondo, M.J.; Vázquez, S.; Riaza, A.; Palenzuela, O.; Sitjà-Bobadilla, A.; Nieto, J.M. Host and environmental risk factors associated with *Cryptosporidium scophthalmi* (Apicomplexa) infection in cultured turbot, *Psetta maxima* (L.) (Pisces, Teleostei). *Vet. Parasitol.* **2009**, *165*, 207–215.
- 30. Baragahare, R.; Becker, J.A.; Landos, M.; Šlapeta, J.; Dennis, M.M. Gastric cryptosporidiosis in farmed Australian Murray cod, *Maccullochella peelii peelii*. *Aquaculture* **2011**, *314*, 1–6.
- 31. Sitjà-Bobadilla, A.; Alvarez-Pellitero, P. Experimental transmission of *Cryptosporidium molnari* (Apicomplexa: Coccidia) to gilthead sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.). *Parasitol. Res.* **2003**, *91*, 209–214.
- 32. Saraiva, A.; Ramos, M.F.; Barandela, T.; Sousa, J.A.; Rodrigues, P.N. *Cryptosporidium* sp. (Apicomplexa) from cultured turbot *Psetta maxima*. *Bul. Eur. Assoc. Fish. Pathol.* **2009**, *29*, 34–36.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).