Parasitol Res
DOI 10.1007/s00436-012-2988-5

SHORT COMMUNICATION

Effect of *Ichthyophthirius multifiliis* parasitism on the survival, hematology and bacterial load in channel catfish previously exposed to *Edwardsiella ictaluri*

Craig A. Shoemaker · Maurício L. Martins · De-Hai Xu · Phillip H. Klesius

Received: 22 November 2011 / Accepted: 25 May 2012
© Springer-Verlag (outside the USA) 2012

Abstract The effect of *Ichthyophthirius multifiliis* (Ich) parasitism on survival, hematology and bacterial load in channel catfish, *Ictalurus punctatus*, previously exposed to *Edwardsiella ictaluri* was studied. Fish were exposed to *E. ictaluri* 1 day prior to Ich in the following treatments: (1) infected by *E. ictaluri* and Ich at 2,500 theronts/fish; (2) infected by *E. ictaluri* only; (3) infected by Ich at 2,500 theronts/fish only; and (4) non infected control. Mortality was significantly higher in fish previously exposed to *E. ictaluri* and then infected by Ich (71.1 %). Mortalities were 26.7 %, 28.9 % and 0 % for fish infected by *E. ictaluri* only, by Ich only and non-infected control, respectively. Quantitative polymerase chain reaction demonstrated the presence of *E. ictaluri* in the brain, gill, kidney and liver of fish infected with *E. ictaluri* regardless of Ich parasitism. At day 8, *E. ictaluri* parasitized fish had significantly more bacteria present in the brain, gill and liver, with no bacteria detected in these organs in the *E. ictaluri*-only treatment, suggesting that the bacteria persisted longer in parasitized fish. Decreased red blood cells count and hematocrit in fish at days 8 and 19 after co-infection suggests chronic anemia. Lymphocyte numbers significantly decreased in all infected treatments versus the non-infected controls at days 2, 8 and 19. Lymphopenia suggests that lymphocytes were actively involved in the immune response. Bacterial clearance was probably influenced by the stress of parasitism and/or the mucosal response induced by ectoparasitic Ich that resulted in the higher mortality seen in the co-infected treatment.

C. A. Shoemaker (✉) · D.-H. Xu · P. H. Klesius
USDA-ARS, Aquatic Animal Health Research Laboratory,
990 Wire Road,
Auburn, AL 36832-4352, USA
e-mail: craig.shoemaker@ars.usda.gov

M. L. Martins
AQUOS-Aquatic Organisms Health Laboratory, Aquaculture Department, Federal University of Santa Catarina (UFSC),
Rod. Admar Gonzaga 1346,
88040-900, Florianópolis, SC, Brazil

Published online: 15 June 2012

Introduction

Aquaculture has expanded in the last 10 years, and the trend of increased production is expected as fish are an important source of protein worldwide (Naylor et al. 2009). Intensification of production has resulted in increased disease due to poor water quality and high stock densities. In intensive aquaculture, the reality of a single pathogen resulting in death loss may be small. More likely many disease agents (parasitic, bacterial, viral and fungal) are present and result in disease. Early work on bacterial, parasitic and viral diseases was mainly single pathogen studies. Cusack and Cone (1985) were among the first suggesting that parasite infection enhanced bacterial diseases of fish. Experimental studies have mostly confirmed their hypothesis (Busch et al. 2003; Labrie et al. 2004; Pylkkö et al. 2006; Bandilla et al. 2006; Xu et al. 2012a,b) and have demonstrated increased mortality in the parasitized/bacteria co-infected fish. Other studies have suggested that parasitism increases host susceptibility due to the stress of parasitism decreasing host resistance (Bowers et al. 2000; Tully and Nolan 2002), thus leading to secondary bacterial infections. Most studies to date have used parasitized fish and/or induced parasitism followed by immersion infection with bacterial pathogens. Limited experimental studies have examined bacterial infection followed by parasitism (e.g., Suomalainen et al. 2005). In their study, rainbow trout (*Oncorhynchus mykiss*), known to be carriers of *Flavobacterium columnare*, were infected with *Diplostomum spathecum* cercariae.

Parasitism by this digenetic trematode did not result in increased disease in *F. columnare* carriers.

Two important diseases of channel catfish aquaculture in the United States and abroad are enteric septicemia of catfish (ESC) and Ich. ESC is caused by the gram negative bacterium *E. ictaluri* and affects channel catfish, *Ictalurus punctatus* of all ages in acute or chronic form (Evans et al. 2011). ESC is responsible for about 50 % of the economic losses to catfish farmers in the US (Shoemaker et al. 2009). Ich is caused by the ciliated ectoparasitic protozoan *Ichthyophthirius multifiliis* (Ich) and is responsible for considerable fish loss although exact monetary values are lacking (Klesius and Rogers 1995; Matthews 2005). In previous studies using the Ich model, we exposed fish to Ich theronts prior to bacterial infection (Xu et al. 2009, 2012a). In these studies, enhanced bacterial invasion and mortality was demonstrated presumably due to the damage caused by the parasite (i.e., enhanced portal of entry).

Hematological assessment is a complementary tool for fish health assessment (Martins et al. 2008). The influence of bacterial (Benli and Yildiz 2004; Martins et al. 2008; Yu et al. 2010) and parasitic infection (Martins et al. 2004, 2011) on hematological parameters has been demonstrated; however, limited work has addressed co-infection studies. The objective of this study was to examine the effect of parasitism by *I. multifiliis* on survival, hematology and bacterial load in channel catfish previously exposed to *E. ictaluri.*

Material and methods

Channel catfish from the same spawn were maintained in holding tanks supplied with flowing dechlorinated municipal water at the United States Department of Agriculture-Agriculture Research Service, Aquatic Animal Health Research Unit, Auburn, AL. Fish obtained from the stock tank were acclimated for 7 days and fed 3–4 % body weight daily (Aquamax Grower 400; PMI Nutrition International, LLC, Brentwood, MO). During the experiment, the means and standard error of water quality were: dissolved oxygen 6.79±0.16 mg/L measured using a YSI 85 oxygen meter (Yellow Springs Instrument, Yellow Springs, OH); pH 6.95±0.04 measured with a Corning 540 pH meter (Corning Incorporated, Corning, NY); ammonia 0.09±0.02 mg/l, nitrite 0.01±0.002 mg/l and hardness 111.1±11.0 mg/l were determined using CEL/890 Advanced Portable Laboratory (Hach, Loveland, CO), and water temperature was 25.5± 0.1 °C, measured daily.

A total of 274 channel catfish, which measured 9.9±0.1 (mean±SE) cm in total length and 8.1±0.2 g in body weight (n=10), were used. Ten catfish were examined and cultured to verify pathogen free status of parasites and bacteria prior to the trial. All fish were negative for *I. multifiliis* and *E. ictaluri*. Fish were distributed into 11 tanks with 24 fish/tank (2–3 tanks/treatment; Table 1) and received the following treatments: (1) infected by *E. ictaluri* and exposed to *I. multifiliis* at 2,500 theronts/fish; (2) infected by *E. ictaluri* and not exposed to *I. multifiliis*; (3) not infected by *E. ictaluri* and exposed to *I. multifiliis* at 2,500 theronts/fish; and (4) not infected by *E. ictaluri* and not exposed to *I. multifiliis* theronts. For fish challenged with *E. ictaluri*, 24 fish were placed into a bucket and immersed in 5 l water with 7.28×10^6 colony forming units (CFU)/ml *E. ictaluri* for 30 min. Fish not exposed to the bacterium were bathed in water with brain heart infusion (BHI) broth for the same time. After challenge, fish were returned to the tank and flowing water was resumed at 0.5 l/min with aeration. One day post *E. ictaluri* infection (DPE), fish were infected by Ich theronts. Water was lowered to 10 l in each tank prior to theront exposure. Ich theronts were added to each tank at 2,500 theronts per fish for the parasitized treatments. The fish were exposed to theronts for 1 h with aeration. The fish in the remaining tanks were not exposed to Ich theronts but kept in 10 l water for 1 h with aeration. Water flow (0.5 l/min) was resumed after 1 h. Three fish per tank were collected 2, 4 and 8 days post *E. ictaluri* exposure for blood samples and determining *E. ictaluri* in different tissues with quantitative polymerase chain reaction (qPCR). The brain, gill, kidney and liver were sampled using aseptic technique and kept at −20 °C for assay. The remaining 15 fish in each tank were monitored for mortality for 19 day post *E. ictaluri* exposure. At day 19, blood samples were collected from three surviving fish per tank.

Edwardsiella ictaluri (AL-93-58) obtained from diseased channel catfish was identified biochemically as described by Panangala et al. (2005). The *E. ictaluri* was incubated in BHI broth at 28 °C in a shaker corresponding to 4.75×10^8 CFU/ml by the standard plate count method at 24 h and used to infect the catfish (as above) and obtain genomic DNA (gDNA). The gDNA from bacterial pellets and fish tissues were extracted using DNeasy tissue kit (Qiagen, Valencia, CA). For standards, 10-fold serial dilutions from 5 ng/μl to 5 fg/μl of gDNA of *E. ictaluri* was made in sterile water or tissue extracts (brain, gill, kidney, liver) as described by Xu et al. (2012a).

Ich was originally isolated from an infected pet fish retailer in Montgomery, Alabama. Ich was maintained by serial transmission on channel catfish held in 50 L glass aquaria. Fish infected with maturing trophonts were euthanized by immersion in 300 mg/L tricaine methanesulfonate (MS-222; Western Chemical Inc., Ferndale, WA) and mature trophonts were gently scraped to dislodge the parasites into an aquarium with aeration, and incubated for 24 h at 24 °C to allow theront development (Xu et al. 2009). Theront concentrations were then quantified in a Sedgewick–Rafter chamber and used to infect the catfish.

Table 1 Cumulative mortality and median days to death (MDD) of channel catfish infected by bacterium *Edwardsiella ictaluri* and *Ichthyophthirius multifiliis*

E. ictaluri (CFU/ml)	Theront Concentration	Tank number	Fish number	Dead number	Mortality (%)	MDD
7.28×10^6	2,500/fish	3	45	32	71.1 ± 12.3^a	6.9^a
7.28×10^6	0/fish	3	45	12	26.7 ± 11.5^b	6.2^a
BHI broth	2,500/fish	3	45	13	28.9 ± 22.6^b	10.0^a
BHI broth	0/fish	2	30	0	0 ± 0^c	NA

Fish was first exposed to *E. ictaluri* and then infected by Ich 1 day post *E. ictaluri* exposure. Mortality (±MSE) was the mean mortality from tanks and observed for 19 days post *E. ictaluri* exposure. Within a given column, means followed by different superscript letters are statistically different ($P<0.05$)

One-step qPCR was performed for detection of *E. ictaluri*. Two *E. ictaluri*-specific primers (forward 5′-ACTTATCGCCCTCGCAACTC-3′ and reverse 5′-CCTCTGATAAGTGGTTCTCG-3′) and a dual-labeled probe (5′-CCTCACATATTGCTTCAGCGTCGAC-3′) described by Bilodeau et al. (2003) were used. The qPCR was performed on an Applied Biosystems 7500 Real-Time PCR System (ABI, Foster City, CA) using Platinum® Quantitative PCR SuperMix-UDG (Invitrogen, Carlsbad, CA). The qPCR mixture in a final volume of 12.5 μl consisted of 1 μl of gDNA from tissue samples, 0.5 μl of 5 mM forward primer, 0.5 μl of 5 mM reverse primer and 10.5 μl of 1x Platinum® Quantitative PCR SuperMix-UDG (Invitrogen). Reactions were run under the following conditions: 50 °C for 2 min, 95 °C for 2 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min (Xu et al. 2012a). The extracted DNA from fish tissue (1 μl) was used as template in qPCR and the DNA concentration in fish tissue was determined from the standard curve (threshold cycle [C_t] values vs. DNA concentration of *E. ictaluri*). Since 1 μl of eluted sample was run in qPCR, the amount of bacterial DNA in each mg of tissue was equal to bacterial DNA concentration (pg/μl)×eluted volume/tissue weight in mg. The bacterial DNA in each mg of tissue was further calculated as genome equivalents (GE)/mg of tissue based on the genome size of *E. ictaluri*=3.8 fg/cell (Bilodeau et al. 2003).

After anesthesia by immersion in 100 mg/l MS-222 solution, the blood was withdrawn from the caudal vein of fish using a 1.0-ml syringe with a drop of 10 % EDTA. Blood smears were stained with Giemsa/MayGrunwald (Rosenfeld 1947) for differential counting of leucocytes. An aliquot was used to determine hematocrit (HTC) (Goldenfarb et al. 1971) and the rest was stored in tubes on ice to quantify the total number of red blood cells (RBC) in a hemocytometer. White blood cells (WBC) were counted in blood extension by the indirect method (Martins et al. 2008).

Mortality, median days to death, GE of *E. ictaluri* in fish tissues and hematological parameters were analyzed with Duncan's multiple range test of the general linear model (GLM) procedure (SAS Institute 1989). *P* values of 0.05 or less were considered significant.

Results and discussion

Mortality was significantly ($P<0.05$) increased in fish that were previously exposed to *E. ictaluri* and then parasitized with Ich (71.1 %; Table 1). Fish infected by *E. ictaluri* alone at 7.28×10^6 CFU ml^{-1} showed 26.7 % mortality, and fish infected by Ich alone without *E. ictaluri* exposure showed 28.9 % mortality. Median days to death were not different among the treatments and no mortality was seen in the non-infected treatment (Table 1).

No *E. ictaluri* was detected by qPCR in the brain, gill, kidney and liver of parasitized or non-parasitized fish that were not exposed to *E. ictaluri* (data not shown). Quantitative PCR analysis demonstrated the presence of *E. ictaluri* in the brain, gill, kidney and liver of fish infected with *E. ictaluri* regardless of parasitism (Fig. 1a–d). No consistent pattern of bacterial load was noted at the early time points in fish tissues between *E. ictaluri* parasitized fish and *E. ictaluri* only fish. Significantly more bacteria (545±34 vs. 207±44 GE/mg) were present in the brain of *E. ictaluri* only infected fish at day 2 (Fig. 1a). However, the liver showed an opposite trend at day 2 with significantly ($P<0.05$) more bacteria present in the *E. ictaluri* parasitized fish (446±169 vs. 1,568±518 GE/mg; Fig. 1b). Bacterial load in all tissues declined at day 4 (Fig. 1a–d). For day 8, *E. ictaluri* parasitized fish had significantly ($P<0.05$) more bacteria present in the brain, gill and liver with no bacteria detected in these organs in the *E. ictaluri* only treatment (Fig. 1a, b and d).

This trial evaluated the effect of Ich parasitism on fish previously exposed to *E. ictaluri.* Fish were exposed to *E. ictaluri* 1 day prior to parasite infection. Hence, parasitism was unlikely to aid bacterial entry into fish. The qPCR results supported this assumption as bacterial loads in brain, gill, kidney and liver between parasitized and non-parasitized catfish were mostly similar. In a previous study, Xu et al. (2012a) exposed catfish to Ich and immersion

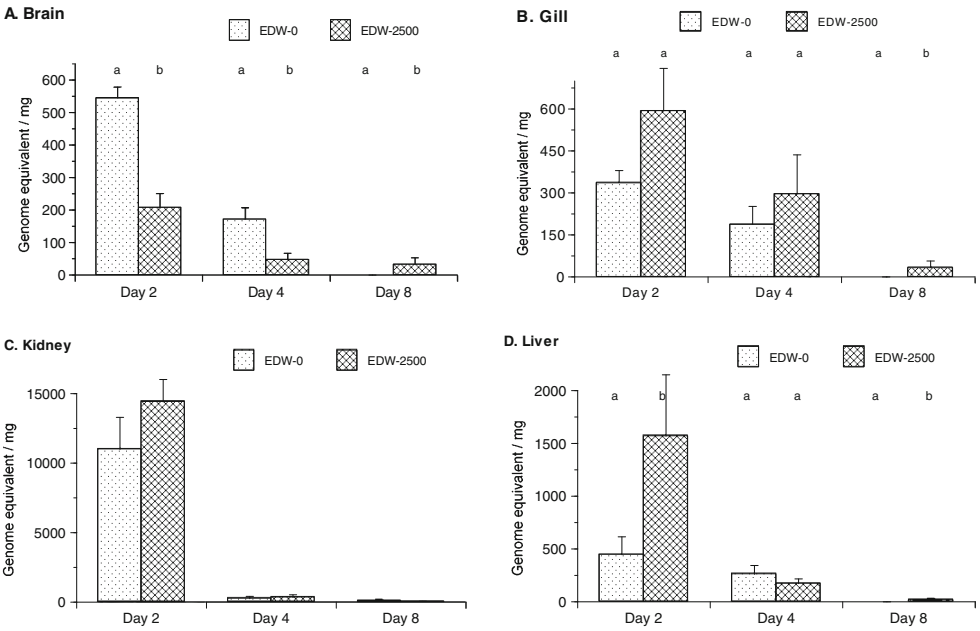

Fig. 1 The average genome equivalent of *Edwardsiella ictaluri* in different tissues (GE/mg) of channel catfish infected by *E. ictaluri* only (EDW-0) and fish co-infected by *E. ictaluri* and *Ichthyophthirius multifiliis* at 2,500 theronts/fish (EDW-2500) were compared at days 2, 4 and 8. Within a given sampling day, means (*bars*) with different superscript letters are statistically different ($P<0.05$)

infected the parasitized fish with *Aeromonas hydrophila*. Results of that study demonstrated that the bacterial load increased significantly in tissues of parasitized fish when compared to non-parasitized fish exposed to *A. hydrophila*, and this resulted in higher mortality of co-infected fish. In the present study, the increased mortality observed in the Ich parasitized fish that had been previously infected with *E. ictaluri* was probably a result of stress caused by the parasite infection influencing the immune response of catfish. qPCR results also suggest that *E. ictaluri* only infected catfish were able to clear the infection from all organs except the kidney at day 8 post *E. ictaluri* exposure. In the *E. ictaluri* parasitized fish, significantly ($P<0.05$) more bacteria was present in brain, gill and liver at day 8, suggesting that parasitism reduced the ability of the fish to clear the bacteria.

RBC did not vary at 2 DPE (Table 2). Significantly ($P<0.05$) decreased RBC count was seen in all infected groups at day 8, with this trend continuing until the end of the experiment (day 19; Table 2). HTC was significantly ($P<0.05$) lower in fish co-infected by *E. ictaluri* and Ich at days 2, 8 and 19 post-*E. ictaluri* infection. Decreased RBC count and HTC in fish at days 8 and 19 after co-infection suggests chronic anemia. Anemia has been demonstrated following bacterial and parasitic infection (Martins et al. 2004; Sabri et al. 2009).

Table 2 Hematological parameters (means±MSE) of channel catfish co-infected by *Edwardsiella ictaluri* and *Ichthyophthirius multifiliis* (Ich)

E. ictaluri concentration	Theronts/fish	DPE	RBC×10^6/μl	HTC (%)	WBC×10^3/μl	Lymp×10^3/μl	Mono×10^3/μl	Neut×10^3/μl
BHI broth	0	2	1.6±0.1^a	43.0±4.2^a	17.5±3.5^a	9.9±1.1^a	3.8±1.5^a	3.7±2.0^a
BHI broth	2,500		1.7±0.1^a	45.3±2.2^a	23.9±5.1^a	1.4±0.3^b	8.1±1.2^b	14.4±4.0^b
7.28×10^6	0		1.5±0.1^a	44.0±1.0^a	14.4±1.7^a	0.9±0.2^b	3.6±0.6^a	9.9±1.1^c
7.28×10^6	2,500		1.9±0.2^a	32.3±1.4^b	14.5±2.9^a	1.1±0.4^b	4.5±0.9^a	8.9±2.1^c
BHI broth	0	8	1.8±0.1^a	40.5±2.8^a	16.3±1.8^a	9.0±0.8^a	5.1±1.0^a	1.9±0.6^a
BHI broth	2,500		0.8±0.1^b	43.7±2.8^a	9.5±1.2^b	1.2±0.2^b	3.8±0.5^a	4.4±0.8^b
7.28×10^6	0		0.9±0.1^b	37.8±1.9^b	32.9±7.8^c	1.9±0.2^b	13.2±2.7^b	9.8±1.8^b
7.28×10^6	2,500		1.2±0.2^b	31.5±0.8^c	43.9±7.4^d	2.5±0.7^b	14.6±2.8^b	26.6±4.7^c
BHI broth	0	19	2.0±0.1^a	47.9±2.0^a	28.2±1.9^a	20.1±1.5^a	6.8±1.0^a	1.1±0.3^a
BHI broth	2,500		1.8±0.1^b	50.5±1.7^a	24.1±2.2^a	8.1±0.9^b	10.0±1.3^a	5.8±1.4^b
7.28×10^6	0		1.6±0.1^b	43.0±1.2^b	91.1±67.1^a	6.8±1.1^c	9.5±1.4^a	6.9±1.6^b
7.28×10^6	2,500		1.4±0.1^c	33.0±1.2^c	27.0±4.2^a	4.2±0.8^d	9.6±0.9^a	13.3±3.6^c

Fish were first infected by *E. ictaluri* for 2 days and then challenged with Ich theronts. Within a given column in each sampling day, means followed by different superscript letters are statistically different ($P<0.05$)

RBC red blood cells, *HTC* hematocrit, *WBC* white blood cells, *Lymp* lymphocytes, *Mono* monocytes, *Neut* neutrophils, *DPE* days post *E. ictaluri* infection

Lymphocyte numbers showed a clear trend of significantly ($P<0.05$) decreasing in all infected groups when compared to the non-infected control group at 2, 8 and 19 DPE. Fish exposed to both *E. ictaluri* and Ich had significantly elevated number of neutrophils at 8 and 19 DPE (Table 2). Lymphopenia and neutrophilia are characteristics of primary response to infection (Garcia et al. 2007; Yu et al. 2010; Martins et al. 2011; Tort 2011) and directly involved on fish immune response (Laing and Hansen 2011). Our results suggest that co-infection resulted in lymphocyte migration from the circulating blood in response to infection as suggested by Garcia et al. (2007) and Olsen et al. (2011).

Most prior studies concerning co-infections in fish have used an opposite approach (i.e., parasitism followed by bacterial infection). Results for the most part have demonstrated that damage caused by ectoparasites to fish skin and gills increase the number of bacteria entering the fish resulting in high mortality. In this study, fish were initially exposed to a bacterial pathogen and then parasitized by Ich. Parasitism of fish previously exposed to *E. ictaluri* also resulted in increased mortality and changes in hematological parameters. Quantitative PCR results demonstrated that the bacteria only exposed fish seemed to clear the infection from most organs by day 8. Ich parasitism results in a local immune response with increased immune gene expression in the skin and gills of fish (Gonzalez et al. 2007; Olsen et al. 2011). The local response may have negatively influenced the immune response to intracellular *E. ictaluri*. The increased mortality and bacterial load in co-infected fish in our study may be due to stress of parasitism and/or the mucosal response induced by Ich.

Acknowledgments The authors thank Jana Mladek, Paige Mumma and Alberta Pink for technical support and The Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Brazil for financial support (no. 201485/2009-5) to M.L. Martins. We also thank Drs. David Pasnik and Ben LaFrentz (ARS-USDA-Aquatic Animal Health Research Laboratory, Auburn, AL, USA) for critical review prior to submission. This work was supported by USDA-ARS CRIS NO. 6420-32000-024D.

References

Bandilla M, Valtonen ET, Suomalainen L-R, Aphalo PJ, Hakalahti T (2006) A link between ectoparasite infection and susceptibility to bacterial disease in rainbow trout. Int J Parasitol 36:987–991

Benli ACK, Yildiz HY (2004) Blood parameters in Nile tilapia (*Oreochromis niloticus* L.) spontaneously infected with *Edwardsiella tarda*. Aquacult Res 35:1388–1390

Bilodeau AL, Waldbieser GC, Terhune JS, Wise DJ, Wolters WR (2003) A real-time polymerase chain reaction assay of the bacterium *Edwardsiella ictaluri* in channel catfish. J Aquat Anim Health 15:80–86

Bowers JM, Mustafa A, Speare DJ, Conboy GA, Brimacombe M, Sims DE, Burka JF (2000) The physiological response of Atlantic salmon, *Salmo salar* L., to a single experimental challenge with sea lice, *Lepeophtheirus salmonis*. J Fish Dis 23:165–172

Busch S, Dalsgaard I, Buchmann K (2003) Concomitant exposure of rainbow trout fry to *Gyrodactylus dejarvini* and *Flavobacterium psychrophilum*: effects on infection and mortality of host. Vet Parasitol 117:117–122

Cusack R, Cone DK (1985) A report on the presence of bacterial microcolonies on the surface of *Gyrodactylus* (Monogenea). J Fish Dis 8:125–127

Evans JJ, Klesius PH, Plumb JA, Shoemaker CA (2011) *Edwardsiella* septicaemias. In: Woo PTK, Bruno DW (eds) Viral, bacterial and fungal infections, 2nd edn. CABI, Wallingford, pp 512–569

Garcia F, Pilarski F, Onaka EM, Moraes FR, Martins ML (2007) Hematology of *Piaractus mesopotamicus* fed diets supplemented with vitamins C and E, challenged by *Aeromonas hydrophila*. Aquaculture 271:39–46

Goldenfarb PB, Bowyer FP, Hall E, Brosious E (1971) Reproducibility in the hematology laboratory: the microhematocrit determination. Am J Clin Pathol 56:35–39

Gonzalez SF, Buchmann K, Nielsen ME (2007) Real-time gene expression analysis in carp (*Cyprinus carpio* L.) skin: inflammatory responses caused by the ectoparasite *Ichthyophthirius multifiliis*. Fish Shellfish Immunol 22:641–650

Institute SAS (1989) SAS/Stat user's guide, 4th edn. SAS, Cary

Klesius PH, Rogers WA (1995) Parasitism of catfish and other farm raised food fish. J Am Vet Med Assoc 207:1473–1478

Labrie L, Komar C, Terhune J, Camus A, Wise D (2004) Effect of sublethal exposure to the trematode *Bolbophorus* spp. on the severity of enteric septicemia of catfish in channel catfish fingerlings. J Aquat Anim Health 16:231–237

Laing KJ, Hansen JD (2011) Fish T cells: recent advances through genomics. Dev Comp Immunol. doi:10.1016/j.dci.2011.03.004

Martins ML, Tavares-Dias M, Fujimoto RY, Onaka EM, Nomura DT (2004) Haematological alterations of *Leporinus macrocephalus* (Osteichthyes: Anostomidae) naturally infected by *Goezia leporini* (Nematoda: Anisakidae) in fish pond. Arq Bras Med Vet Zootecnia 56(5):640–646

Martins ML, Mouriño JLP, Amaral GV, Vieira FN, Dotta G, Bezerra AJM, Pedrotti FS, Jerônimo GT, Buglione-Neto CC, Pereira G Jr (2008) Haematological changes in Nile tilapia experimentally infected with *Enterococcus* sp. Brazil J Biol 68:631–637

Martins ML, Shoemaker CA, Xu D-H, Klesius PH (2011) Effect of parasitism on vaccine efficacy against *Streptococcus iniae* in Nile tilapia. Aquaculture 314:18–23

Matthews RA (2005) *Ichthyophthirius multifiliis* Fouquet and ichthyophthiriosis in freshwater teleosts. Adv Parasitol 59:159–241

Naylor RL, Hardy RW, Bureauc DP, Chiua A, Elliott M, Farrelle AP, Forstere I, Gatlin DM, Goldburg RJ, Hua K, Nichols PD (2009) Feeding aquaculture in an era of finite resources. Proc Natl Acad Sci 106:15103–15110

Olsen MM, Kania PW, Heinecke RD, Skjoedt K, Rasmussen KJ, Buchmann K (2011) Cellular and humoral factors involved in the response of rainbow trout gills to *Ichthyophthirius multifiliis* infections: molecular and immunohistochemical studies. Fish Shellfish Immunol 30:859–869

Panangala VS, van Santen VL, Shoemaker CA, Klesius PH (2005) Analysis of 16 S–23S intergenic spacer regions of the rRNA operons in *Edwardsiella ictaluri* and *Edwardsiella tarda* isolates from fish. J Appl Microbiol 99:657–669

Pylkkö P, Suomalainen LR, Tiirola M, Valtonen ET (2006) Evidence of enhanced bacterial invasion during *Diplostomum spathaceum* infection in European grayling, *Thymallus thymallus* (L.). J Fish Dis 29:79–86

Rosenfeld G (1947) Corante pancrômico para hematologia e citologia clínica. Nova combinação dos componentes do May–Grünwald e

do Giemsa num só corante de emprego rápido. Mem Inst Butantan 20:329–334

Sabri DM, El-Danasoury MAE-H, Eissa IAE-M, Khouraiba HM (2009) Impact of henneguyosis infestation on hematological parameters of catfish (*Clarias gariepinus*). Int J Agric Biol 11:228–230

Shoemaker CA, Klesius PH, Evans JJ, Arias CR (2009) Use of modified live vaccines in aquaculture. J World Aquacult Soc 40:573–585

Suomalainen L-R, Tiirola MA, Valtonen ET (2005) Influence of rearing conditions on *Flavobacterium columnare* infection of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis 28:271–277

Tort L (2011) Stress and immune modulation in fish. Dev Comp Immunol 36:1366–1375

Tully O, Nolan DT (2002) A review of the population biology and host–parasite interactions of the sea louse *Lepeophtheirus salmonis* (Copepoda: Caligidae). Parasitology 124:16–182

Xu D-H, Shoemaker CA, Klesius PH (2009) Enhanced mortality in Nile tilapia *Oreochromis niloticus* following coinfections with ichthyophthiriasis and streptococcosis. Dis Aquat Organ 85:187–192

Xu D-H, Pridgeon JW, Klesius PH, Shoemaker CA (2012a) Parasitism by protozoan *Ichthyophthirius multifiliis* enhanced invasion of *Aeromonas hydrophila* in tissues of channel catfish. Vet Parasitol 184:101–107

Xu D-H, Shoemaker CA, Klesius PH (2012b) *Ichthyophthirius multifiliis* as a potential vector of *Edwardsiella ictaluri* in channel catfish. FEMS Microbiol Let 329:160–167

Yu JH, Han JJ, Park SW (2010) Haematological and biochemical alterations in Korean catfish, *Silurus asotus*, experimentally infected with *Edwardsiella tarda*. Aquacult Res 41:295–302