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Parasitic Helminth Infection in Tropical Freshwater Fishes of Commercial Fish Farms, in Morelos State, Mexico

[1,2]D. Hernández-Ocampo, [3]R.F. Pineda-López, [2,4]J.T. Ponce-Palafox and [5]J.L. Arredondo-Figueroa
[1]Facultad de Ciencias Naturales, Posgrado en Recursos Bióticos, Universidad Autónoma de Querétaro, Querétaro, México
[2]Laboratorio de Bioingeniería Acuícola, Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Morelos, México
[3]Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Querétaro, México
[4]Centro Nayarita de Innovación y Transferencia Tecnológica, Posgrado CBAP, Escuela Nacional de Ingeniería Pesquera, Universidad Autónoma de Nayarit, Tepic, Nayarit, México
[5]Posta Zootécnica, Centro de Ciencias Agropecuarias, Universidad Autónoma de Aguascalientes, Aguascalientes, México

Abstract: The principal aim of this study was realize a helminthological register of the species founded in ornamental fishes cultured in three fish farms and wild fishes existent in the effluents close to these farms in Morelos State, Mexico and an analysis and comparation of the infection values and their relative importance of helminth species invaders associated to ornamental fish industry and their manifestation in wild fishes close to farms. The helminth parasites of freshwater fishes of commercial fish farms, in Morelos State, Mexico were analysed. A total of 419 organisms of six freshwater fish species were collected in three fish farms and in small streams adjacent, in rainy and dry seasons. A total of 40 528 helminth were collected; the metacercarian of the flatworm *Centrocestus formosanus* affected largest number of host species. There were high levels of infection in all fish for this fluke, specifically for the Mexican molly, *Poecilia sphenops*; it was also found in the native poeciliid fish the Asian tapeworm *Botriocephalus acheilognathi*, although with lower values in the parameters of infection. A non-deteriorated environment allowed complete the life cycles of parasites, permitting the establishment and maturation in various native and exotic fishes favoured the lack of host specificity of helminths and allogeneic generalists.

Keywords: Aquaculture, farm, helmints, native and exotic fish, parasites

INTRODUCTION

The introduction of exotic aquatic species has been identified as one of the more critical environmental risk affecting native species, aquatic environments and biodiversity. This phenomenon is related with the extinction of 54% of the world native aquatic fauna, the 70% of North American fishes and 60% of Mexican fishes (IMTA *et al*., 2007). The invasive exotic species displace native species by direct competition, depredation, diseases and parasites transmission, habitat modifications, alteration of trophic levels structure and their biophysical conditions (Contreras-MacBeath *et al*., 1998; Contreras-Balderas *et al*., 2008).

In the Morelos State, Mexico, are known more than 25 fish species, 69% of them have been introduced and exploited with two main purposes; as human consumption and in the ornamental industry (Contreras-MacBeath *et al*., 1998; Conabio and Uaem, 2004) and for this reason the aquaculture farms and close small streams are considered as a source to establishment and dispersion of exotic parasites. Some helminths studies realized in small streams of Morelos State in species such as the Godeid *Ilyodon whitei* and the Cyprinid *Hybopsis boucardi* contributed with preliminary data about the helminths parasites introduced in fresh water fishes (Salgado-Maldonado, 2006).

In Mexico at the present time, 262 helmints parasites of nominal species had been reported, from which, 194 are native and 18 introduced. The more common species are *Centrocestus formosanus* and *Bothriocephalus acheilognathi*. The metacercarian of *C. formosanus* have been reported in 12 families of fishes and 59 species, the transmission to fishes in through an intermediary host, in this case the snail *Melanoides tuberculata* and their maturation is achieved in birds as definitive host. The adult helminth more dispersed is the Asian cestod *B. acheilognathi* that

Corresponding Author: J.T. Ponce-Palafox, Centro Nayarita de Innovación y Transferencia Tecnológica, A.C Posgrado CBAP, Escuela Nacional de Ingeniería Pesquera, Universidad Autónoma de Nayarit, Tepic, Nayarit, México

have been registered in seven families and 59 species of freshwater fish in streams and culture ponds (Salgado-Maldonado, 2006).

In great proportion parasites are disseminated and introduced to new localities for the movement of infected host (Kennedy, 1976). However, in Mexico the anthropogenic interest over any scientific opinion has been the main factor for the introduction and establishment of helminth species in new localities (Scholz and Salgado-Maldonado, 2000; Scholz *et al*., 2001; Salgado-Maldonado and Pineda-López, 2003). In the Morelos state the uncontrolled introduction of exotic fishes had caused the presence of high rates of infection parameters in cultured fishes and in small streams and bodies of water. The principal aim of this study was realize a helminthological register of the species founded in ornamental fishes cultured in three fish farms and wild fishes existent in the effluents close to these farms in Morelos State and an analysis and comparation of the infection values and their relative importance of helminth species invaders associated to ornamental fish industry and their manifestation in wild fishes close to farms.

MATERIALS AND METHODS

This study was conducted from September 2005 to May 2006. The first fish farm was Atlacomulco, in municipality Jiutepec, Morelos state, México, located at 18° 54´04" N and 99° 12´19" W at 1,415 m about sea level (masl); the source of water of this farm is Chapultepec spring (an effluent of the Apatlaco River); the second was Cuatlita farm, in municipality Tetecala, Morelos sate, México, located in 18° 42´06" N and 99° 22´22" W at 938 masl; the source of water is a channel derivate of the Chalma river (Tembembe River) and the third is El Potrero Farm, in municipality Cuautla, Morelos state, México, located in 18° 49´59" N and 98° 59´25" W at 1,277 masl; the source of water is an small branch stream of the Cuautla river (Cuautla River).

A total of 419 fishes were collected and analysed from the rainy season of 2005 to dry season of 2006. Six ornamental fish cultured were included representing five families of exotic fishes cultured in Morelos; golden carp, *Carassius auratus* and koi carp *Cyprinus carpio koi* (Cyprinidae); dwarf gourami, *Colisa lalia* (Belontidae); butterfly tetra, *Gymnocorymbus ternetzi* (Characidae); sailfin catfish, *Liposarcus multiradiatus* (Loricaridae); guppie, *Poecilia reticulate* (Poeciliidae). Besides, three wild fish of small streams close to farms were also included; ten spotted live-bearer, *Cnesterodon decenmaculatus*, Mexican molly, *Poecilia sphenops* (Poeciliidae) and tilapia, *Oreochromis sp*., (Cichlidae). Fishes were translated alive to the laboratory and examined for ecto and endo helminth parasites. The parts of the body examined were: skin, fins, mouth, anus, branches, digestive tube, liver, gonads, visceral cavity and muscles, inside the 24 h after collected using conventional methods proposed by Salgado-Maldonado (1979) and Vidal-Martínez *et al*. (2002). The prevalence, abundance and mean intensity infection were calculated according by Bush *et al*. (1997).

RESULTS

A total of 40 528 helminth were collected and identified. Six species were recognized, three of them are introduced species *Centrocestus formosanus*, *Botriocephalus acheilognathi* and *Cichlidogyrus sclerosus*; one was considered as native species *Eustrongylides* sp. and two of them can be considered in uncertain category *Gyrodactylus* spp and *Dactylogyrus* spp According of the nature of specificity host of the parasites were considered as generalists *C. formosanus* an *B. acheilognathi*, because they infect different families of host; *C. sclerosus*, *Eustronglydes sp*., *Gyrodactilus spp* and *Dactylogirus spp* were considered as specialist, due their distribution, development and reproduction is restricted to one unique species, genus or host family, with an affinity to parasite determined corporal regions. *C. formosanus*, *B. acheilognathi* and *Eustrongylides sp*., were considered as alogenetic species because they parasite fishes or other aquatic vertebrates, as intermediate host and sexually maturates in birds and mammals (definitive host). The autogenic species finish their life cycle inside of aquatic ecosystem and were represented in this study by *C. sclerosus*, *Gyrodactylus spp* and *Dactylogyrus spp*.

In the Table 1 is shown the infection helminth parameters in five fish species in Atlacomulco farm examined between the rainy and dry seasons of 2005 and 2006, respectively.

Four species of parasites were identified and 5 198 helminth were collected. Monogenea were presented a genus level, but they were characterized as morphospecies. A higher infection parameter was observed in *C. carpio koi* where the trematode *C. formosanus* presented a prevalence of 92% and an abundance of 179.6±209. The minor prevalence was founded in *C. auratus* with 50% and 4.5±5.5, respectively. C. *formosanus* was the more important parasite in function of the infection parameters and the host range infected, moreover, is considered a serious forecast for the population of freshwater fishes, due they produce cysts on branches filaments of numerous species of freshwater fishes that interfere with the respiratory process (Scholz and Salgado-Maldonado, 2000). This parasite did not was registered in the exotic guppy *P. reticulata* and in this fish only was collected the monogenean *Gyrodactylus spp*. with a prevalence

Table 1: Helminth collected in five species in Atlacomulco fish farm, Morelos, Mexico

Date/season	Fish species	Helmint species	Parasite host	Number of helminth	Prevalence (%)	Abundance ±S.D.	Mean infection intensity ±S.D.
26/VIII/2005	Cck (n = 25)	*C. formosanus*	23	4491	92	179.6±209	195±211
(rainy)		*Gyrodactylus* spp	3	11	12	0.44±1.5	3.6±2.9
		Dactylogyrus spp	8	8	32	2.3±4.5	7.1±5.3
7/IX/2005	Lmu (n = 25)	*C. formosanus*	20	102	80	4.1±3.3	5.1±2.9
(rainy)		*Gyrodactylus* spp	5	8	20	0.32±0.7	1.6±0.6
		Dactylogyrus spp	10	15	40	0.6±3.3	1.5±2.9
8/V/2006	Cau (n = 30)	*C. formosanus*	15	136	50	4.5±5.5	9±5.30
(dry)		*Gyrodactylus* spp	27	134	90	4.5±3.8	5±3.70
		Dactylogyrus spp	26	150	86.6	5±3.40	5.7±3.0
20/II/2006	Pre (n = 22)	Gyrodactylus spp	12	30	54.5	1.3±1.6	2.5±1.31
(dry)							
24/V/2006	Psp (n = 22)	*C. formosanus*	12	111	54.5	5±7.5	9.2±7.8
(dry)		*Eustrongylides* sp.	2	2	9	0.09±0.29	1±0

Cck: *Cyprinus carpio koi*; Lmu: *Liposarcus multiradiatus*; Cau: *Carassius auratus*; Pre: *Poecilia reticulata*; Psp: *Poecilia sphenops*; C: *Centrocestus*; S.D.: ±standard deviation; n: Sample

Table 2: Helminth collected in six species in Cuautlita fish farm, Morelos, Mexico

Date/season	Fish species	Helminth species	Parasite host	Number of helminth	Prevalence (%)	Abundance ±S.D.	Mean infection intensity ±S.D.
19/IX/2005	Col (n = 25)	*C. formosanus*	25	17599	100	704±250	704±250
(rainy)		*Gyrodactylus* spp	5	20	20	0.8±2.2	4±3.5
25/IX/2005	Lmu (n = 26)	*C. formosanus*	21	307	81	11.8±3.5	15±3.9
(rainy)		*Gyrodactylus* spp	9	49	35	1.9±22	5.4±23
22/II/2006	Psp (n =21)	*C. formosanus*	21	412	100	19.6±17.3	19.6±17.3
(dry)		*Dactylogyrus* spp	1	2	4.8	0.09±0.43	2±1.41
		Eustrongylides sp.	2	3	9.5	0.14±0.48	1.5±0.71
22/II/2006	Cde (n = 30)	*C. formosanus*	28	775	93.3	25.8±30.5	27.7±30.8
		Eustrongylides sp.	1	1	3.3	0.03±1.18	1
22/II/2006	Ore (n = 17)	Cichlidogyrus sclerosus	3	11	17.6	0.64±1.54	3.6±1.52
17/IV/2006	Cau (n = 30)	*C. formosanus*	29	2318	96.6	77.3±83.3	80±83.5
(dry)		*Dactylogyrus spp*	6	49	20	1.6±3.7	8.2±4.2

Col: *Colisa lalia*; Lmu: *Liposarcus multiradiatus*; Psp: *Poecilia sphenops*; Cde: *Cnesterodon decenmaculatus*; Ore: *Oreochromis* sp.; Cau: *Carassius auratus*; C: *Centrocestus*; S.D.: ±standard deviation; n: Sample

Table 3: Helminth collected in four species in El Potrero fish farm, Morelos, Mexico

Date/season	Fish species	Helminth species	Parasite host	Number of helminth	Prevalence (%)	Abundance ±S.D.	Mean infection intensity ±S.D.
21/VII/2005	Cau (n = 16)	*C. formosanus*	12	1761	75	68±141	147±146
(rainy)		*Gyrodactylus* spp	13	159	81	9.9±7.3	12±6.1
		Dactylogyrus spp	8	423	50	26±47	53±56
8/XI/2005	Cau (n = 15)	*C. formosanus*	14	1.022	93	68±42	73±39
(rainy)		*Gyrodactylus* spp	11	77	73	5±4.7	7±4.1
		Dactylogyrus spp	14	168	93	11±42	12±39
8/III/2006	Cau (n = 30)	*C. formosanus*	30	12,634	100	421.1±238.7	421.1±238.7
(dry)		*Gyrodactylus* spp	23	499	76.6	16.6±31.5	21.7±34.6
		Dactylogyrus spp	29	2.553	96.6	85.1±131.2	88±132.5
28/IV/2006	Psp (n = 30)	*C. formosanus*	27	430	90	14.3±21.6	15.9±22.3
(dry)		*B. acheilognathi*	2	3	6.7	0.1+0.4	1.5±0.7
		Eustrongylides sp.	6	11	37	0.4+0.8	1.8±0.7
21/IV/2006	Gte (n = 25)	*C. formosanus*	24	1057	96	42.3±49.1	44±49.4
(dry)		*Dactylogyrus spp*	25	5602	100	224±158	224±158
2/V/2006	Lmu (n = 30)	*C. formosanus*	3	19	10	0.63±2.04	6.3±2.5

Cau: *Carassius auratus*; Psp: *Poecilia sphenops*; Gte: *Gymnocorymbus ternetzi*; Lmu: *Liposarcus multiradiatus*; C: *Centrocestus*; S.D.: ±desviación estándar; n: Simple

of 54.5% and an abundance of 1.3±1.6. The larval stage of the nematode *Eustrongylides sp*., only was founded in *P. sphenops* outside of the farm.

The results of helminth parasites founded in Cuautitla Farm are shown in Table 2. The infection parameters of three exotic fish's *C. lalia*, *L. multiradiatus* and *C. auratus*, moreover, of three species living in small streams outside the farm *Oreochromis spp*, *P. sphenops* and *C. decenmaculatus* are shown. Five species of parasites were reported and the metacercarian *C. formosanus* was the most relevant and was present in all freshwater fishes analysed except in *Oreochromis spp* that only presented the exotic monogenean *Cichlidogyrus sclerosus* a specialized parasite of this fish species. From 21 546 collected helminth, *C. formosanus* represented the 99.4% in the six species. The dwarf gouramy *C. lalia* presented higher prevalence with the 100% and an abundance of

704±250 and similarly to the first farm analysed the nematode larvae *Eustrobylides sp*., only was present in *P. sphenops* and *C. decenmaculatus* fishes living in small streams outside the farm.

In the Potrero Farm were analysed four freshwater fish species, three of them exotic; golden carp, *C. auratus*, butterfly tetra, *G. ternetzi* and the sailfin catfish, *L. multiradiatus* and a wild fish living in a small stream outside the farm the Mexican molly, *P. sphenops* (Table 3). Five species of parasites were collected; the metacercarian *C. formosanus* was the more frequent with higher prevalence and abundance in *C. auratus* in the rainy and dry seasons in 2005 and 2006. This Cyprinid besides presented *Gyrodactylus spp* in fins and *Dactylogyrus spp* in branches during the dry season (Table 3). *B. acheilognathi* was present only in *P. sphenops* collected in a small stream outside the farm, also was collected during the dry season the nematode larvae *Eustrongylides sp*., that showed higher prevalence (37%) and abundance (0.4±0.8). *G. ternetzi* presented higher infections of *Dactylogyrus spp* with 100% of prevalence and the same average abundance and intensity (224±158). For the other side, the sailfin catfish *L. multiradiatus* showed only *C. formosanus* parasite, but with lower infection parameters, compared with the other infected fishes.

DISCUSSION

The majority of helminth founded in freshwater fishes in Mexico was introduced join with other fishes of nutritional and ornamental importance. The metacercarian of the trematode *C. formosanus* (Nishigori, 1924) and the Asiatic cestod *B. acheilognathi* (Yamaguti, 1934), exhibit a wide distribution in different basins and states of Mexico, affecting many native and exotic freshwater fishes (Scholz and Salgado-Maldonado, 2000; Salgado-Maldonado and Pineda-López, 2003). At the present time, *B. acheilognathi* is considered the helminth with more successful in the word, due practices of aquaculture propitiate indiscriminate introduction and dispersion of the Asiatic carps, their original host. The fishes are the definitive host of this cestod that acquire the parasite when they consume copepods that are the intermediate host. The copepods are infected when they consume coracidiae larvae. However, in this study the infected intensity of this cestod can be considered low, because only was observed in *P. sphenops* outside of the farms in a small stream. In contrast, farm fishes did not presented this parasite; this can be explained by the intensive management methods used in farms and that they do not produce Chinese carps, moreover, in intensive aquaculture fishes are submitted to stressing environment fluctuations such as high densities, heavy transportation, use of drugs, poor water quality and a long production chain including different process as production, distribution and commercialisation (Negrete *et al*., 2004).

The metacercarian of *C. formosanus* affected a higher number of host species. The dispersion is propitiated by the snail *Melanoides tuberculata*, the first intermediary host that is present in different bodies of water and small streams, favouring their dispersion and establishment. The differences of proportions of infected fishes could be influenced by the number of samples, but the mechanisms of production of ornamental fishes is dynamic mechanism and they have short life cycles and then, did not was possible obtain the desired numbers of parasites. However, their variation in farms can be analysed with golden carp *C. auratus* that was collected in El Potrero Farm. In the rainy season carps presented a prevalence of 93% and an abundance of 68±42 of helminth and in dry season a prevalence of 100% and an abundance of 421.1±238.7, respectively and no were observed significant differences in the prevalence, but there were significant differences in abundance between both seasons. Moreover, this trematode presented higher infection of parameters in the three farms.

The helminth registered in native freshwater fishes in the Balsas System River indicate the presence of *B. acheilognathi* in *Astyanax fasciatus* (Caracidae), *Archocentrus nigrofasciatum* and *Cichlasoma istlanum* (Cichlidae), *Hybopsis boucardi* (Cyprinidae) and *Heterandria bimaculata*, *Poecilia reticulate*, *Poecilia sphenops* and *C. decenmaculatus* (Poecilidae) (Salgado-Maldonado, 2006). In this system river the metacercarian of *C. formosanus* have been reported in *Astyanax fasciatus* (Carcidae), *A. nigrofasciatum* (Cichlidae) and *Xiphophorus helleri*, *P. sphenops* and *C. decenmaculatus* (Poecilidae). These data coincide with the helminths founded in the present study; further include new reports of these parasites in ornamental fishes.

The nematode larvae *Eustrongylides sp*., was reported in *A. nigrofasciatum*, *P. gracilis* and *P. sphenops* in the Amacuzac River in Morelos State (Salgado-Maldonado, 2006). In this study these larvae presented lower infection parameters and was founded exclusively in the wild fishes *P. sphenops* and *C. decenmaculatus*. There is scarce information about the biological cycle of this parasite, but is know that eggs are big and present three larvae stages and the definitive host is a bird (Ramírez-Lezama and Osorio-Sarabia, 2002).

The allogeneic species have higher capacity of colonization and for this reason have a wide distribution, between the factors favouring the establishment of these helminth species as parasites of freshwater fishes are the presence of ichtyophagous birds, however it is important to point out that farms presented a low proportion of allogeneic species, in part because in the intensive culture ponds they use anti-

birds nets or greenhouses to avoid the depredation of fishes and the dispersion of these parasites is restricted.

The helminthological fauna of fishes includes also autogenic species. Data showed the presence of helmints anthropogenic introduced join with allochthonous fish species. The monogenean *Cichlidogyrus sclerosus*, *C. tilapiae* and *C. dossoui* are well established in Mexico and they present a high relative prevalence and abundance in cichlids and are considered specific of their African hosts and does not exist evidences of infection in native fishes in the regions where they were introduced and neither the American monogenean infecting African cichlids (Jiménez-García *et al*., 2001). In this investigation was studied the tilapia *Oreochromis sp*., that was infected by *C. sclerosus* although with lowers values of prevalence and abundance. Several species not identified of *Gyrodactilus* have been reported in tilapia (*Oreochromis* sp.), golden carp (*Carassius auratus*), common carp (*C. carpio*) and ten-spotted live-bearer (*C. decenmaculatus*) and *Dactylogyrus* in Nile (*Oreochromis niloticus* and *Oreochromis* sp.,) and grass carp (*Ctenopharyngodon idella*) in ponds and farms in Morelos State (Salgado-Maldonado, 2006). In this study the infection parameters of this monogenean was founded in golden carp (*C. auratus*) in Atlacomulco and El Potrero farms showing high variability possibly due to the management practices of aquaculture, frequent changes of water quality and the use and administration of drugs to guaranties the health of culture fishes.

Thilakaratne *et al*. (2003) reported that *Gyrodactylus* and *Dactylogyrus* presented higher rates of infection and are common in ornamental fishes destined to exportation, in Sri Lanka. In this sense the main sanitary problems in Morelos State fish production, are related with parasitizes diseases such as protozoan, gyrodactylosis and centrocestosis, due the absence of preventive practices, indiscriminate mobilization of organisms and high densities in ponds (Rodríguez-Gutiérrez *et al*., 2005).

Small and constant changes in fish production systems that are common in intensive cultures, generate a considerable stress in the homeostatic mechanisms, resulting in an increase disease susceptibility and infections of a wide variety of parasites (Scholz, 1999), moreover, supported by the results found in this research the exotic fish culture is characterized by the differences in prevalence and abundance of helmints parasites and spite of the high diversity of fish cultures in farms in Morelos State, was not found a similar diversity of parasites helmints species, however, high infection values were registered in the three farms caused by the invader trematode *C. formosanus* in the Mexican molly *P. sphenops* population and Asian invader *B. acheilognathi* even though with lower values of infection. The results founded in this study emphasize an alarming situation generated by the grade of invasion of this helminth parasites in the ornamental fish culture in Morelos State, caused by an erroneous government politics allowing the indiscriminate introduction of exotic fishes without an strict sanitary control, justified by an economical and social interests that had propitiated the introduction of exotic helmints and their transfer to native species, causing health deterioration and a poor quality production of ornamental fishes, ignoring the possible adverse impacts on aquatic ecosystems and the native biota.

From the ecology point of view the presence of invader helminth in the three localities studied are agree with Bauer (1991) concerning the establish and colonization of a new locality for a parasite species is inversely related with the cycle life complexity and the probability of the success of the colonization and related with the similarity of the inter-fauna degree between the sites of origin and introduction, moreover, the importance of helminth parasites is consistent with the arguments of Aguilar-Aguilar and Salgado-Maldonado (2006) who consider that sites with high richness of helminth species make evident an acceptable environmental quality, allowing the intermediary and definitive host and their existence in adequate densities population completing their cycles of life, moreover, the lack of host specificity of the generalist and allogeneic helminth make possible the establishment and maturation in diverse exotic and native freshwater fishes.

CONCLUSION

The majority of helminth founded in freshwater fishes in Mexico was introduced join with other fishes of nutritional and ornamental importance. There were high levels of infection in all fish for this fluke, specifically for the Mexican molly, *Poecilia sphenops*; it was also found in the native *poeciliid* fish the Asian tapeworm *Botriocephalus acheilognathi*, although with lower values in the parameters of infection. A non-deteriorated environment allowed complete the life cycles of parasites, permitting the establishment and maturation in various native and exotic fishes favoured the lack of host specificity of helminths and allogeneic generalists.

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