Revista Digital del Departamento
El Hombre y su Ambiente
ISSN: 2007-5782

2013. Vol.1 (1): 12-19

Assessment of *Escherichia coli* and *Salmonella arizona* as opportunistic pathogens in angelfish (*Pterophyllium scalare* Lichtenstein 1823) aquaculture.

[1]Monroy-Dosta MC, *, [1]Negrete-Redondo MP, [2]Romero-Jarer JM, [1]Torres-Lima P.

[1]Universidad Autónoma Metropolitana, Unidad Xochimilco. Laboratorio de Producción de Alimento Vivo. Calzada del Hueso 1100, Col. Villa Quietud, C. P. 04960 Del. Coyoacán. Tel. (55) 4065-6136

[2]Universidad Nacional Autónoma de México. Instituto de Ciencias del Mar y Limnología, C.P.04510, Coyoacán, México, D.F.

Email: monroydosta@hotmail.com

ABSTRACT

In centers of aquaculture production of angelfish (*Pterophyllum scalare)* in Xochimilco, Mexico City, massive mortality (90% of the production) was recorded. The dead fish manifested atypical clinical signs, such as: hemorrhagic eyes, pale gills, weight loss, anorexia and the nervous system affected. Furthermore, after necropsy, internal hemorrhage was not observed in any of the cases at different stages of the diseases, like those of bacteria known as ichthyopathogen. Not knowing the etiological relationship between analyzed fish and involved pathogens, samples of kidneys from fish that manifested the clinical signs aforementioned. The samples were seeded in selective agars. The bacteria were identified using API-20E and API-20NE systems. Different genera of the family *Enterobacteriaceae*, such as *Escherichia*, *Salmonella* were identified. The infection capacity of *E. coli* and *S. arizona* in clinically healthy angelfish was tested. In order to define the etiological relationship between the isolated bacteria and their host, four groups of fish were kept under the same conditions than those of angelfish production tanks: 28°C, pH 7, 5 mg mL^{-1} of dissolved oxygen and 0.3 ppm of nitrates and nitrites. Infection bacteria doses (10^6 and 10^5 cuf mL^{-1}) of *E. coli* and *S. arizona* were inoculated intramuscularly to the fish. Multivariate analysis with Jackknifed test and discriminant analysis were applied to establish the existence of influential variables, in order to define which organs were affected depending on the pathogen and the dose used, and to establish which signs and lesions were more frequent, for which the statistical SYSTAT 9.0 pakage was used. In this work, we established that *E. coli* and *S. arizona* are opportunistic pathogens and when environmental conditions change, they show as virulent and provoke an acute disease in susceptible species to environmental stress.

Key words: ornamental fish aquaculture, experimental infection, atypical pathology

INTRODUCTION

One of the main branches of aquaculture is the production of a great variety of aquatic organisms of commercial value, in which freshwater ornamental fish are included; these are of special interest because they are an important source of income for rural groups dedicated to its culture (Arredondo and Ponce 1998) and, on the other hand, promoting development of research lines for to the study of these organisms (SEMARNAT 2003). Among ornamental cultured fish, cichlids probably represent the most important group. Ornamental fish fans have strong preference for the culture of angelfish (APPOEM AC 2001).

One of the main problems faced by ornamental aquaculture is disease. Fish pathologies are a great problem in commercialization and production that affects economic and social development of ornamental fish producers in different countries of the world (FAO 2001). According to reports from producers in Mexico City, losses due to infections associated with environmental stress reach 90% of the total production, therefore the importance of prevention, diagnosis and control of these diseases (OIE 2003). Epidemiology of almost all viral and bacterial fish diseases are related with stress factors, such as temperature changes, salinity, pH, decrease in dissolved oxygen, excessive handling or increase in the levels of particulate matter in water (Quintana 2001).

Cultured aquatic organisms health may be conceived as physiological wellbeing. The absence

Receive: Febraruary 1st 2013. Accepted: May 1st 2013. Published: July 1st 2013.

Revista Digital del Departamento
El Hombre y su Ambiente
ISSN: 2007-5782

2013. Vol.1 (1): 12-19

of disease is an essential element for physical wellbeing. The three major factors to unchain an epizootic are: species susceptibility to the pathogen present, virulence of this and adverse environmental conditions that cause stress in cultured fish. Likewise, it is the balance of these factors which determines health condition; therefore, alterations in any of these can unchain an infection (Sniesko 1973).

It is important to consider that aquaculture production systems success depends in great part on the availability of food, especially live food, which is essential in fish larvae because they can only feed from very small organisms that contain necessary nutriments that allow them to reach commercial size in a short time (Lim 2003). Besides, a malnourished organism is very susceptible to environmental variations and thus to diseases (Padrós y Furones 2002, Auró y Ocampo 1999, Dehasque et al. 1989, Negrete y Romero 1998).

In Mexico City, ornamental fish production centers, atypical infectious outbreaks have been detected, such is the case of three angelfish production centers in Xochimilco, where there was a failure in the power supply that altered for eight hours the physicochemical conditions of the culture pond (first, temperature dropped to 18ºC and then rose to 32ºC, consequently, the levels of dissolved oxygen were affected); simultaneously, massive mortality of angelfish was recorded. Fish showed diverse signs of infection that had not been previously recorded, such as hemorrhagic eyes, lethargic swimming, weight loss and very pale gills.

At necropsy, internal fish organs showed no hemorrhages, they were only pale. Likewise, enterobacteria were isolated directly from kidneys, among them: *Escherichia coli* and *Salmonella arizona*, which are reported as human pathogens, but not as an ornamental fish pathogen, for which the etiological relationship between these bacteria and host (angelfish) is still unknown.

In the presence of infectious enterobacteria outbreaks with different clinical profiles to the already studied and caused by fish bacteria, the objective of the present study was to establish the etiological relationship between *E. coli* and *S. arizona* and host *P. scalare*.

MATERIAL AND METHODS

Fish samples were obtained and taken from culture ponds with a spoon net from an angelfish production center located in Xochimilco, Distrito Federal, where production mortality was manifested with signs and lesions of atypical disease. They were anesthetized with tricaine methane sulphonate ($0.1\ gL^{-1}$) for one minute. Dissection was performed making a cut with a sterile scalpel, from the operculum to the base of caudal fin. A kidney sample was taken from the dead fish with a sterile bacteriological loop and seeded in agar plates of thiosulfate-citrate-bile salt- sucrose (TCBS) and brain heart infusion agar (BHI), incubating it for 24 hours at 35ºC (APHA 1992).

Colonies were purified through successive reseeding in BHI agar plates. Gram stain was performed, observing cellular morphology through an optic microscope, finally pure isolated strains were identified using commercial identification systems API-20E and API-20NE, following product manufacturer instructions (Analytical Profile Index 1992 y Analytical Profile Index 1997).

Fifty fish (*P. scalare*), in juvenile phase, were introduced in a culture tank, previously equipped in the laboratory to keep them acclimatized under the same culture conditions as in production centers; that is: 28ºC, pH 7, 5 mg/mL of DO and 0.3 ppm of nitrates and nitrites. Behavior was observed for eight days, replacing any fish that would show any sign of infection or "abnormal" behavior (gasping, anorexia, exophthalmia, erratic swimming, desquamation or hemorrhagic eyes).

With the aim to establish if the fish have had previous contact with the pathogens to be inoculated, *E. coli* and *S. arizona*, immunoglobulin titre was carried out from a subsample of fish in observation, 0.2 mL of blood serum were extracted from four fish and agglutination reactions were performed against the aforementioned bacteria using parallel dilution technique (Bradshaw 1973).

Simultaneously, inocula were prepared by seeding with a 3 mm calibrated loop, seeding three times each of the *E. coli* and *S. arizona* pure cultures, in vial flasks with 50 mL of BHI broth. It was left to incubate in water bath with agitation for

Receive: Febraruary 1st 2013. Accepted: May 1st 2013. Published: July 1st 2013.

Revista Digital del Departamento
El Hombre y su Ambiente
ISSN: 2007-5782

2013. Vol.1 (1): 12-19

24 hours at 35ºC (Michel 1980). After 24 hours dilutions from 1:10 (10^7, 10^6, 10^5, 10^4) were made, for which 5 mL from the original inoculum were extracted and were added to a vial with 45 mL of the same medium to obtain a 10^7 dilution, and thus consecutively until dilution 10^4 was reached. From each dilution, 0.1 mL was extracted with an automatic pipette and was seeded homogenously on BHI agar using an angled glass rod; later, plates were incubated at 35°C for 24 hours (Michel 1980); then, colony forming units (cfu mL^{-1}) were counted to ensure strain viability and determine the number of cfu mL^{-1} inoculated to each individual (Arévalo et al. 2003).

Ten fish (*P.scalare*) were distributed in each of the aquariums displayed in the following way: aquarium 1 was left as control, where fish were inoculated with 0.8% steril saline solution, in order to observe the experimental stress effect; aquariums 2 and 3 were used for inoculated fish with *E. coli* with infectious doses (10^6 and 10^7); aquariums 4 and 5 were allocated for inoculated fish with *S. arizona* with infectious doses (10^6 and 10^5 cfu mL^{-1}), respectively. Fish were inoculated intramuscularly, under the dorsal fin and above the lateral line, with a sterile insulin needle. The inoculum was previously dosed according to the size of the organism, 1 mL $100g^{-1}$ of fish (Michel 1980).

From this moment and until the organisms died, changes in behavior, clinical signs and lesions caused by infection were recorded each hour. Necropsy was performed in dead fish, in order to determine presence or absence of lesions in internal organs and be able to take a direct kidney sample that was seeded in BHI agar plates and incubated at 35°C for 24 hours. The obtained colonies were purified, Gram stain were performed and identified by the aforementioned systems (Dehasque et al. 1989, Analytical Profile Indexb1992).

In order to establish if the fish that overcame the infection produced antibodies against *E. coli* and *S. arizona*, the immunoglobulin agglutination reaction was once more performed as cited before (Bradshaw 1973).

For the analysis of the obtained data of the clinical profile of the fish, with regard to pathogens and inoculated dose, a Jackknifed test and a discriminate analysis were carried out, with the objective to establish the existence of influential variables, that is, which organs were, affected (Ruiz y Deturnewlle 2000). For this, the statistical SYSTAT 9.0 package was used, by which the existence of significant differences between the groups of the measured variables were analyzed, and the influential variables were defined, in other words, which organs were more affected depending on the pathogen and the dose, or which signs were most strongly presented among the following: color, skin, scales, fins and tail, mouth, gills, eyes, body, appetite, behavior, swim, digestive tube, kidney, liver, swim bladder, gall bladder, heart and gonads, according to degree of lesion: 0 when no lesion or damage, 1 when slight damage was observed, 2 when mild damage and 3 severe damage.

RESULTS

Fish (*P. scalare*) cultivated in the production center where the present study was carried out, showed infection signs such as hemorrhagic eyes, anorexia, weight loss, eroded fins, pale gills and slow swimming. At necropsy, absence of bleeding in different internal organs was observed. These organisms died within the first 48 hours from the first infection manifestation signs. From the first kidney samples taken from these fish, families *Vibrionaceae*, *Aeromonaceae* and *Pseudomonaceae* were isolated, predominating *E. coli* and *S. arizona* belonging to the *Enterobacteriaceae* family (Table 1).

Table 1. Bacterial charge (cfu mL^{-1}) with different bacterial species isolated from kidney of infected *P. scalare.*

Sample	cfu mL^{-1}	Identified species
1	135	*Salmonella arizona*
2	77	*Aeromonas hydropphila*
3	35	*Pseudomonas cepacea*
4	60	*Aeromonas salmonicida*
5	45	*Vibrio fluvialis*
6	43	*Vibrio parahaemolyticus*
7	170	*Escherichia coli*
8	56	*Vibrio vulnificus*
9	85	*Pseudomonas* sp.
10	74	*Samonella* sp.

Receive: Febraruary 1st 2013. Accepted: May 1st 2013. Published: July 1st 2013.

Revista Digital del Departamento
El Hombre y su Ambiente
ISSN: 2007-5782

2013. Vol.1 (1): 12-19

Before the study, samples of blood extracted from two individuals chosen at random from the *P. scalare* group kept in acclimatization period in the laboratory, showed titres of 1:60 µl and 1:80 µl.

Fish from the control group that were inoculated with saline solution showed general signs and lesions of infection: edema, flaking, anorexia, nervous behavior; however, 30 hours after inoculation, the signs disappeared and the organisms recovered (Group 2).

In the group inoculated with 10^6 cfu mL^{-1} of *E. coli*, signs started 24 hours after inoculation. These signs were: skin discoloration, hemorrhagic eyes, anorexia, nervous behavior, pale gills, bristle scales, slow and erratic swimming. At necropsy, practiced to the two organisms that died within the following 24 hours after inoculation, the most relevant finding was absence of bleeding from internal organs; nevertheless, the digestive tract was observed lacerated, with bad odor and disseminated abdominal liquid. Fish that survived to the infection recovered their initial health condition after 48 hours of being inoculated (Table 2).

The experimental group inoculate with 10^5 cfu

Table 2. Diagnostic characterization of *P. scalare* fish inoculated with: 0.8% sterile saline solution and different doses of *E. coli*, *S. arizona* and *A. hydrophila.*

Inoculum	Saline solution	*E. coli* 10^6 cfu mL^{-1}	*E. coli* 10^5 cfu mL^{-1}	*S. Arizona* 10^6 cfu mL^{-1}	*S. Arizona* 10^5 cfu mL^{-1}	*A. hydrophila* 10^6 cfu mL^{-1}
Signs						
Skin	Edema	Discoloration	Discoloration	Discoloration Presence of mucus	Discoloration	Presence of mucus
Scales	Bristle	*	*	Bristle	*	Flaking
Fin and tale	*	*	*	Bristle	*	Hemorrhagic
Mouth	*	*	*	*	*	Constant gasping
Gills	*	Pale	Pale	Pale	Pale	Hemorrhagic
Eyes	Exophthalmia	Hemorrhagic	Hemorrhagic	Hemorrhagic	Hemorrhagic	Exophthalmia
Body	*	Thinning	Thinning	Thinning	Thinning	Swollen
Appetite	Anorexia	Anorexia	Anorexia	Anorexia	Anorexia	Anorexia
Behaviour	Nervous	Nervous	Nervous	Nervous	Nervous	Nervous
Swim	Normal	Slow and erratic	Slow and erratic	Slow and erratic. Passive	Slow and erratic	Espasmodic
Necropsy						
Digestive tube	*	Lacerated	Lacerated	*	*	Hemorrhagic
Kidney	*	*	*	*	*	Discarded
Liver	*	*	*	*	*	Hemorrhagic
Gall bladder	*	*	*	*	*	Discarder
Swim bladder	*	*	*	*	*	Broken
Heart	*	*	*	*	*	*
Gonads	*	*	*	*	*	*
Other	*	Without internal hemorrhage Two fish dead	Without internal hemorrhage Two fish dead	*	*	Internal hemorrhage, all fish dead

* No signs were manifested

Receive: Febraruary 1st 2013. Accepted: May 1st 2013. Published: July 1st 2013.

mL^{-1} of the same pathogen, initiated the infectious process 24 hours after inoculation with skin discoloration, bristled scales, anorexia, body weight loss, passive behavior, slow swimming and pale gills. Immediately after the two fish died, necropsy was done, where lacerated digestive tract was observed, but there was no hemorrhage of the internal organs or gills. After 5 days, fish started to recover until reaching their initial health condition (Table 2).

The clinical profile initiated 24 hours after the provoked infection with 10^6 cfu mL^{-1} of *S. arizona*, was characterized by skin discoloration, bristled scales, hemorrhagic eyes, weight loss, anorexia, pale gills, slow and irregular swimming and passive behavior. However, fish were slowly recovered their initial health condition. All overcame the infection (Table 2).

Fish inoculated with 10^5 cfu mL^{-1} of *S. Arizona* showed signs of mild infection three days after being inoculated, with changes such as discoloration, pale gills, hemorrhagic eyes, weight loss, anorexia, slow and irregular swimming and nervous behavior. After four days, behaviour and health of the organisms was reestablished. All survived (Table 2).

Immunoglobulin titers obtained from fish that survived to experimental infection recorded in all cases, an increase in titre levels: 1:2 280 µl and 1:2 560 µl.

When performing statistical analysis based on the canonical discriminant function (standardized variance), it was considered that the four most important variables are signs and lesions showed in gills, skin, eyes and fish swimming (Table 3).

The discriminant analysis showed that all information is explained with two discriminant factors: factor 1 indicates that treatment with *E. coli*, without considering concentration, is discriminated by paleness of the gills, while for *S. arizona* without considering concentration of the doses, is discriminated by hemorrhagic eyes. In the control case, it is discriminated by skin lesions. With regard to *A. hydrophila* 10^6 cfu mL^{-1}, it was observed that it is discriminated by hemorrhagic gills (Fig. 1).

Considering discriminant factor 2, *S. arizona*, without regard to the dose, is discriminated by skin discoloration and fish erratic swimming; *E. coli*, by skin discoloration and hemorrhagic eyes, while the control group is discriminated by the presence of exophthalmia. *A. hydrophila* 10^6 cfu mL^{-1} was discriminated by skin edema and exophthalmia (Fig. 1)

Table 3. Standardized and non-standardized canonical correlation values with the discriminant function (variance) of signs and lesions observed in fish inoculated with 106 y 10^5 cfu mL^{-1} of *E. coli y S. Arizona*.

Signs y Lesions	Factor 1	Factor 2
Values	13.661	1.631
Canonical correlation	0.965	0.787
Accumulated proportion	0.790	0.884
Canonical discriminant function		
Correlation	4.734	2.174
Fins and tails	0.167	1.039
Mouth	0.088	0.340
Gills	8.471	5.497
Eyes	7.117	6.447
Body	-0.429	-1.725
Appetite	-0.368	-0.205
Behaviour	0.169	0.459
Swim	4.163	2.324
Digestive tube	0.188	0.500
Liver	-0.533	-1.222
Gall	-1.090	-4.191
Swim bladder	0.123	-1.581
Heart	WDV	WDV
Gonads	-0.757	-0.359

WDV=without discriminant value

DISCUSSION

The bacterial charge identified from fish kidney samples, is the result of bacterial contamination in culture system, especially in the case of *E. coli* and *S. arizona*, bacteria associated with human settlements and agricultural waste (Negrete y Romero 1988, Austin y Austin 2003).

Immunoglobulin titres done previous to the experiment revealed that fish had been in contact

Receive: Febraruary 1st 2013. Accepted: May 1st 2013. Published: July 1st 2013.

Revista Digital del Departamento
El Hombre y su Ambiente
ISSN: 2007-5782

2013. Vol.1 (1): 12-19

with the inoculated pathogens, although the titres were considered low (Negrete et al. 2004).

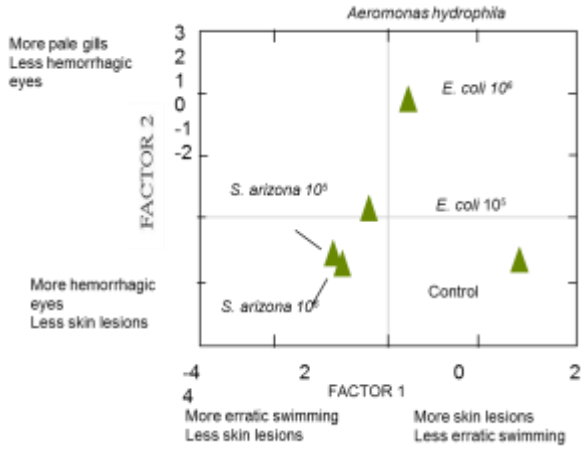

Fig.1. Discriminant analysis of the clinical profile presented by *P. scalare*, inoculated with 10^6, 10^5 cfu mL^{-1} of *E. coli;* 10^6, 10^5 cfu mL^{-1} of *S. arizona*; control group and 10^6 cfu mL^{-1} of *A. hydrophila*.

The manifestation of infection signs in the control group was the consequence of mechanical shock implied by the injection of saline sterile solution. It is considered that the manifestation of these signs that disappear in a short period of time, was due to the immune response decrease in fish because of the experimental stress in presence of bacteria that form part of fish normal flora (Michel 1980).

Until now, *E. coli* and *S. arizona* bacteria have not been recorded as pathogenic in bibliography of *P. scalare* fish; however, in this study it was observed that when inoculating infective doses 10^6 and 10^5 cfu mL^{-1} of these bacteria to *P. scalare* healthy fish, Koch postulates were fulfilled, since by isolating these bacteria from sick fish from the production center, they proliferated in pure cultures for several generations; likewise, when inoculating these bacteria to *P. scalare* fish the typical disease was reproduced. Of the individuals that died, the inoculated pathogens were recovered by taking samples from kidney and seeding them in BHI agar plates. Finally, the fish that survived the infection generated antibodies against the pathogen; this was proven by the elevation of titres levels against *E. coli* and *S. arizona.* The aforementioned shows that such bacteria have the capacity to cause infection. In both cases there was absence of internal organ hemorrhage, signs and lesions such as thinning, pale gills, hemorrhage at the base of the eyes, eroded fins and slow swimming. Only 20% of the inoculated fish with *E. coli* died, which indicates that the bacteria is not strictly pathogen for *P. scalare* fish. It has been recorded (Souza et al. 2000) that *E. coli* possesses a high level of evolution due to its genes or plasmids transference capacity through "lateral transference"; besides, one strain of this bacterium can reach different levels in the host's organs. With this, it is defined that this bacterium can be found in different forms of infection depending on the penetration degree that has in the host's organism (Munro 1982).

Infective doses of inoculated pathogens is equivalent to 50,000 cfu mL^{-1} and 60,000 cfu mL^{-1}, which in comparison with infective doses used by other authors in bacteria like *A. hydrophila* and *V. fluvilais* in *C. uratus*, is a high dose, for which it was determined that the pathogens studied had low virulence, since there is an inverse relation with regard to cfu mL^{-1} quantity of the inoculum (Walter y Plumb 1990).

The diagnostic characterization does not coincide with the reported in studies carried out by other authors (Walter y Plumb 1990, Cottral 1986), with fish pathogenic bacteria such as *A. hydrophila* and *A. salmonicida*, which are of ichthyopathogenic interest that produce a great variety of diseases in fish, in which infectious signs are characterized by general hemorrhagic septicemia in all internal organs, besides presence of furunculosis on the body, associated with ulcerative dermonecrosis, hemorrhagic gills, spasmodic swim, nervousness and exophthalmia that were not observed in experimental fish (Negrete et al. 2002). This is due to the inoculated bacteria, which are not strictly pathogen to ornamental fish, but opportunistic pathogens that only actuate when environmental conditions are adverse, as proven in previous studies (Walter y Plumb 1990), in which the evident stress effect of water temperature on the host is expressed, where there are strong changes in its physiology that

Receive: Febraruary 1st 2013. Accepted: May 1st 2013. Published: July 1st 2013.

may cause more severe or different clinical profiles, according to bacteria number and diversity present in that moment (Cottral 1986)

Water temperature is a very important factor in the development of many fish diseases, because they are poikilothermic and their body temperature is always similar to their aquatic environment. Fish physiological responses, including defense mechanisms, depend on body temperature. Therefore, the result of a fish infection caused by a pathogen is significantly affected by water temperature (Walter y Plumb 1990). In such way that when there is a brisk change of environmental temperature, a strong thermal shock that submits fish to an acute environmental shock, opportunistic bacteria found in the system are favoured.

Continuous power supply failures presented in the studied aquaculture center altered environmental conditions of the culture, changing water temperature and as a consequence the pH, nitrites and nitrates and DO levels, which propitiated a pathogen-host-environment unbalance, as posed by Snieszko (1973), this situation altered the ratio health-disease when opportunistic pathogens such as *E. coli* and *S. arizona* together with a susceptible host in an environment that does not fulfills handling condition and adequate exploitation (Negrete y Romero 1998).

The statistical analysis applied showed differences between clinical profiles presented by fish from 10^6 and 10^5 cfu mL^{-1} of *E. coli* and *S. arizona* inoculation compared to the control group inoculated with saline solution, and the profile presented by *P. scalare* fish with 10^6 cfu mL^{-1} of *A. hydrophila.* The discriminant analysis showed that, in the case of *E. coli*, the inoculated dose concentration was important, because used at a dose of 10^5 cfu mL^{-1}, fish presented erratic swimming, but at a higher concentration (10^6 cfu mL^{-1}) the disorder was manifested by pale gills; however, the disorder caused by *S. arizona* is reflected in a greater degree by hemorrhagic eyes and skin discoloration, without regard to the inoculated doses. Skin and eyes are affected in greater degree in the control group; in this case there was skin edema and exophthalmia, but there was no skin discoloration or hemorrhage in eyes as it was observed in the experimental group. Comparing experimental groups with fish inoculated with 10^6 cfu mL^{-1} of *A. hydrophila*, it was observed that it is totally discriminant because of the lesions showed in gills. However, these lesions are totally different to the observed in the experimental groups, in which gills were pale, while with *A. hydrophila* hemorrhage was manifested. With regard to the observed lesions in the experimental group there was hemorrhage, whereas with *A. hydrophila* there was exophthalmia. The aforementioned shows the differences between the clinical profiles presented by the inoculated fish with different doses of *E. coli, S. arizona* at 10^6 cfu mL^{-1} and *A. hydrophila,* because this is a bacterium that causes acute profiles associated with ulcerative dermonecrosis and furunculosis (Austin y Austin 2003).

CONCLUSSIONS

According to the results it is possible to conclude that enterobacteria *E. coli* and *S. arizona* are opportunistic pathogenic bacteria of Angelfish (*P. scalare*), hence environmental conditions must be kept under strict surveillance to avoid infectious outbreaks of opportunistic pathogens that when finding favorable environmental conditions can cause virulence and putting the organisms in culture at risk (Austin y Austin 1987).

BIBLIOGRAPHY

Analytical Profile Index. 1992. *Enterobacteriaceae* and other Gram negative Bacteria, 9th. Edition Bioumerioux, Francia.

Analytical Profile Index. 1997. *Enterobacteriaceae* and other Gram negative Bacteria, 9th. Edition Bioumerioux, Francia.

APHA. 1992. Standard methods for examination of water and waste water. 17th. Ed. Washington, American Public Health Association. Washington D. C. pp 583-598.

APPOEM A. C. 2001. Asociación de Productores de Peces de Ornato del Estado de Morelos. Diagnóstico de la Producción en las granjas asociadas a la APPOEM. A. C. Secretaria de Desarrollo Agropecuario de Morelos. SAGARPA.

Receive: Febraruary 1st 2013. Accepted: May 1st 2013. Published: July 1st 2013.

Arévalo Z, AM Clavijo, DM Rolo, M Alvarez, D Conroy y D Infante. 2003. Isolation of *Vibrio cholerae* from lisas and tilapias in Venezuela. Revista de la Sociedad Venezolana de Mirobiología. 2.

Arredondo J y J Ponce. 1998. Calidad del agua en acuicultura conceptos y aplicaciones. México, D. F. AGT. Editor, S. A.

Auró A y CL Ocampo. 1999. Diagnóstico del estrés en peces. Revista Veterinaria México. 337-344.

Austin B y DA Austin. 1987. Bacterial fish pathogen deseases in formes and wild Fish. Ellis Horwood Ltd. London. 364

Austin B y DA Austin. 2003. The pathogenicity of *Vibrio* tipe strains to salmonids. Department of Biological Sciences. Herriott-Walt University of Richartown. Edinburgh, EH144AS. UK International Symposium of Aquatic Animal Health.

Bradshaw LJ. 1973. Laboratory Microbiology. Second edition. W.B. Saunders Company. Philadelphia.

Cottral GE. 1986. Microbiología Veterinaria. Ediciones científicas de la prensa mexicana, México.

Dehasque M, T Wolf, P Candevra, P Coutteau y P Sorgeloos. 1989. Control of bacterial input through the live feed in marine fish hatcheries. Aquaculture. 249-256.

FAO, 2001. Estadísticas de la producción de acuicultura. FAO, Circular de pesca Nº 815.

Lim LC. 2003. Recent development in the application of live feeds in the Freswater ornamental fish cultura. Aquaculture. 246.

Michel C. 1980. A standardized of experimental furunculosis in rainbow trout (*Salmo gairdneri*). Canadian Journal of Fisheries Aquaculture Scienses. 746-750.

Munro ALS. 1982. The pathogenesis of bacterial diseases of fish. En Robles R-Journal of Microbial Diseases of Fish. Academia Press, London, pp 131-149.

Negrete RP y JJ Romero. 1998. Estudio cualitativo de las condiciones sanitarias de producción y manejo de granjas acuícolas en los estados de México y Morelos. Revista Hidrobiológica. 43- 54.

Negrete RP, JJ Romero y FJL Arredondo. 2004. Capacidad de *Vibrio fluvialis* (Lee, 1981) para producir infección en pez dorado (*Carassius auratus*,L.) Revista Veterinaria México. 35.

Negrete RP, JJ Romero, JD Valencia y LG Villegas. 2002. Bacterosis por estrés ambiental en granjas acuícola rurales del Estado de México. Sociedades. Rurales. Producción y Medio Ambiente. 49-58.

OIE. 2003. Código Sanitario para los animales acuáticos. Organización Mundial de Sanidad Animal. Capítulo 1.5.1.

Padrós F y MD Furones. 2002. Patología bacteriana en piscicultura. Annual Review of Fish Diseases. 34:13.

Quintana A. 2001. Riesgo sanitario asociado al comercio de animales acuáticos. Revista Acuatic.14: 34-59.

Ruiz RT y CY Deturnewlle. 2000. El análisis discriminante en estudios biológicos. Revista Biología, Facultad de Biología. Universidad de la Habana Cuba

SEMARNAT. 2003. Estado actual de la acuacultura. SECRETARIA GOBIERNO DE MORELOS. Boletín informativo. Cuernavaca Morelos. Ed. SEMARNAT.

Souza V, A Castillo y A Eguiarte. 2000. The evolutionary ecology of *Escherichia coli.* American Science. 90.

Snieszko SF. 1973. Recents advances in scientific knowledge and development pertaining to diseases of fish. C. A. Bradly and C.E. Cornelius, ed.

Walter G y R Plumb. 1990. Environmental stress and bacterial infection in channel cats fish *Ictalurus punctatus*. Reviews Journal of Fish Biology. 177-185.

Receive: Febraruary 1st 2013. Accepted: May 1st 2013. Published: July 1st 2013.