

THE SUSCEPTIBILITY OF NILE TILAPIA TO *AEROMONAS HYDROPHILA* INFECTION POST HEAT EXPOSURE

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SUMMARY

The current experiment was conducted to spot light on the impact of thermal heat stress on the susceptibility of Nile tilapia to *Aeromonas hydrophila* infection. Four different groups of Nile tilapia (*Oreochromis spp.*) were used as follows: the first and the second groups were exposed to sudden rising of the water temperature up to [Upper Critical Limit UCL] (37-40°C) and by the end of the 48 hours the 1st group was challenged with *A. hydrophila*. The third group was kept under normal temperature (19-21°C) (comfort zone), and by the end of the 48 hr it was challenged with the aforementioned Bacteria.

The forth group was held as control under normal temperature, clinical signs, PM lesions and case fatality % were recorded for both the heat stressed fish and the challenged group.

Whole citrated blood samples were collected in successive timing intervals from each group separately, namely, 1 hr., 24 hr. 48 hr and 72 hr. post heat stress. Some biochemical parameters were measured as glucose, total protein, and cortisols. Blood films were prepared to calculate the Heterophil, Lymphocyte, (H/ L ratio). The histopathological features of the different organs of the heat stressed Nile tilapia were discussed

It was found that fish under warm-water stress at (UCL) 37-40°C suffered from abnormal behaviors; expressed obvious clinical signs as well as increased mortalities .PM recorded severe congestion in internal organs. The plasma glucose increased directly post stress then declined till 48 hours. Values of Plasma cortisols and total plasma protein (TPP) of stressed fish were varied along the course of the experiment.

The histopathological features of internal organs

revealed severe hyperemia and congestion in the different organs.

It is recommended that correct transportation and gradual acclimation of fish in its new habitats must be done, as on the national levels, proper disposing of industrial waste water mainly of the power stations and the cooling and refining water systems moustatae should be considered to avoid fish health risk and economic losses induced by increased diseases susceptibility and outbreaks with *A. hydrophila* infection.

INTRODUCTION

Fish are constantly exposed to bacteria and usually only succumb to an infection after having exposed to prolonged periods of stress. Environmental factors may act as stressors and can predispose a fish to bacterial disease.

Genus *Aeromonas* are constant microbiota of fresh reservoirs where they together with other microorganisms play the part of natural bio-filter and promote with self purification. They are necessarily presents in normal microflora and hydrobionats inhabiting fish reservoirs (Kompanots et al ; 1992).

A. hydrophila infections are known as red sore disease (Esch et al., 1976; Overstreet and Howe, 1977) is reported to be the cause of high mortality

in reared *O. mossambicus*, *O. niloticus* and *Tilapia zillii*, in the Philippines (Lio-Po et al., 1983). The course of the disease usually run in an acute manner, clinical conditions, associated with systemic infections, result in mortalities within 24-48 hours .Tank-reared, two-week-old fry died at a rate of 15% daily , affected fish were heavily pigmented, with petechial haemorrhages. In more chronic type of clinical conditions, eroded fins occur as well as skin lesions and sluggish swimming (Roberts and Sommerville, 1982 and Lio-Po et al., 1983).The mortalities were between 10% and 70% among cultured fish. Haemorrhagic septicemia has been reported from pond cultured tilapia (*O. niloticus*) in Japan, (Miyazaki et al., 1984). *Aeromonas* species were found in greatest number in summer.

There are some potential risk factors associated with the main diseases of fish such as season and water temperature (Ortega et al; 1995). Among physical thermal stressed fish mortality was 80% due to *Aeromonas* . (Noga, 1996).

In intensive fish culture, mortalities were observed in late spring and early summer due to *A. hydrophila* infection (Faisal et al., 1989).

There are many indicators that the stress response is variable and flexible in fish, in line with the great diversity of adaptation, that enable these animals to live in a large variety of aquatic habitats (Wendelaar Bonga, 1997).

Environmental stress factors which influences fish immune system may be natural include sea-son, temperature, salinity and photoperiod and so-cial stress factors such as crowding and hierarchy .Both artificial and natural environmental stresses appear to suppress immune functions. They sup-press components of both the innate and adaptive arms of the immune system (Bly et al;1997).

The heated discharges of the industrial wastes can drastically alter the ecology of a water source.

Discharge of thermal waters from power stations and cooling water reservoirs can result in a con-siderable increase in the dissemination of patho-gens and count of opportunistic microorganisms which may present a serious environmental health risk (Solovykh, et al;1998; Suzdaleva, 2001 and Nasser et al; 2003).

Understanding the dynamic of the aquatic envi-ronment and its role in fish health is imperative to management of infectious disease. Fish respond in concert with, and quickly to environmental changes that affect diseases susceptibility and overall general health. Fish diseases generally oc-cur seasonally and tend to fluctuate with tempera-ture changes, presence of young susceptible fish in a population and environmental conditions that affect immunity and natural resistance (Plumb, 1999).

Plasma cortisol is from the initial and known pa-rameter of stress that is usually measured because of its easiness and availability (Alsop et al;(1999). Therefore the aim of this work is to spot light on the following:

1. The impact of the physical heat stress on Nile tilapia.
2. The susceptibility of the stressed Nile tilapia to *A. hydrophila* infection.
3. The study of some blood parameters and his-topathology of the heat stressed Nile tilapia.

MATERIALS AND METHODS

1) Fish

A total number of 140 from Nile tilapia fish were obtained from Al Abbasa fish farm, Al Sharkia Governorate, grouped into 4 groups each 35.The main weight of fish was ranged 40 ±5 gm.. Every fish group was kept in glass aquarium(80x 40 x 30 cm³) supplied with de-chlorinated tap water The fish groups were left for acclimation for 2 weeks, according to (Tort, et al 2003).

2) Environmental conditions .

The aquaria were supplied with sufficient aeration using electric pumps to maintain a level of 6.5 ±0.2 mg/l dissolved oxygen, temperature was ad-justed to be 19°C, pH value was measured weekly by electric digital pH-meter for a value of 7.1 ±0.1 and a hardness of 150mg/l as calcium carbo-

nate. The values were measured according to the American Public Health Association (APHA 1992). The water was exchanged to maintain good water quality according to Alabaster and Li-oyd, (1980), all fish were fed daily on the 35% protein pelleted diet at a rate of approximately 3% of their average body weight according to Jauncey and Ross (1982).

3) Experimental design

The fish were grouped in to 4 separate groups as follows: the first and the second groups were subjected to addition of poured boiled water on the

side of the aquarium, the third and the fourth groups were left untreated as control as shown in table no. (1). The initial temperature and pH of water were recorded. Water temperature intended to be between 37-40°C as upper critical temperature (UCT) where its temperature increased from 19-27-33-37-40°C within 40 minutes and left for 1 hr. at 35°C then declined after adding dechlorinated 19°C tap water, from 35-33-30-26 for 45 minutes. During adding boiled water the fish behaviors were monitored and recorded. Morbidity and mortality percentages were recorded regarding time and temperature table no. (2).

Table (1): The Number of fish, the grouping and the heat stress pattern for each group of Nile Tilapia fish:

Group No.	Number of fish	Treatment
G1	35	Heat stress (37-40°C)
G2	35	Heat stress (37-40°C)
G3	35	Control to be challenged 19°C
G4	35	Control 19°C

Table (2): Water temperature zones along the course of Stressed Nile tilapia.

Variables	C.T.C	TLV of T.C	UCT.C	Thermal decline .C
	19-21	27-33	37-40	37-33-30
Time spent minutes	10	10	20	45

C.T.C.: Comfort Temperature in Centigrade (Comfort zone).

TLV .C: Threshold Limit Value In Centigrade (Threshold zone).

UCT.: Upper Critical Temperature In Centigrade (Critical zone).

4) Biochemical and hematological studies

Whole citrated blood were collected separately from the morbid fish according to (Noga, 1996). The plasma were separated from each sample and collected separately for measuring glucose using Stanbio-kits following Trinder, 1969, cortisol using active cortisols Enzyme immunoassay, EIA (DSL-10-2000) kit. (Cherwell Innovation centre, 2003) and total plasma protein according to (Weichsel baun, 1964). Blood smears were made and stained with Geimsa stain for differential leukocytes count and estimation of Heterophil/Lymphocyte ratio (Hesser, 1960).

5) Histopathological studies:

For histopathological examination tissue samples were taken from brain, liver, spleen, and intestine of heat stressed fish the samples were fixed in 10% buffered neutral formalin solution , processed by standard paraffin methods, sectioned at 4-5 um and finally stained with Haematoxylin and Eosin (Bancroft et al., 1996).

6) Microbial challenge

By the end of the 48hr. post experimentation, the intended groups of Nile tilapia (the 1st and the 3rd groups) were anesthetized according to (Post,1987) challenged by IP injection of 0.2 ml of 24 hr broth culture of *A. hydrophila* containing approximately 10^6 CFU/ml according to Lafrentz et al (2002). Fish mortalities and development of clinical abnormalities were recorded for 7 days post challenge.

RESULTS AND DISCUSSION

1-Clinical alteration and behavioral changes observed in different groups of heat stressed Nile tilapia

The Nile tilapia exposed to heat stress showed prominent signs as shown in plate no. (1) including: asphexia manifested by mouth breath with increase opercular movement and accumulation near the aerators (fig. 3), this can be attributed to the fact that the water temperature is in reversal correlation with its oxygen content, as the temperature increases in water the level of oxygen diminish so fish try to seek any source of oxygenation (Noga, 1996). Group of fish escaped toward the cold aquarium wall away from the hot spot (fig. 1), surfacing was the beginning behavior of the motion and end by stagnation on the bottom of the aquaria , The behaviors of escaping from the water by jumping out of water was very obvious. This behavior might be due to either response of hot water threaten fish life or seeking colder water with more oxygen. Skin over pigmentation (darkness) this is attributed to the aggregation of the melano macrophages from the circulation to the skin layers as a response to the stress (Post, 1987), exophthalmia (fig. 5), increase mucus secretion and oozing blood from the gills (fig. 4), tail serration and discoloration(fig. 2), however the production of glucose with stress assists the animal by providing energy substrates to tissues such as brain , gills and muscles in order to cope with the increased energy demand.

(Iwama, et al; 1999).The morbidity, mortality and case fatality% were (100% morbidity) and 57% case fatality as shown in table no. (3).PM, features of stagnant and dead fish revealed congested gills with blood oozes (fig.4), congested internal organs particularly, heart and spleen and kidney, slight pale liver (fig. 6).

of cortisol and TPP confirmed the harsh effect of hot water on plasma parameters as well as flexibility of fish to cope environmental stresses. There are many indicators that the stress response is variable and flexible in fish (Barton and Iwama 1991), in line with the great diversity of adaptation, that enable these animals to live in a large

Table (3): Morbidity, Mortality and Case fatality of heat-stressed Nile tilapia.

Group	Morbidity		Mortality		Case fatality	
	Number	%	Number	%	Number	%
G1, G2	35/35	100%	20/35	57%	20	57
G3, G4	0	0%	0	0%	0	00

2) Plasma glucose, cortisol and total protein post heat stress.

The readings in table (4) showed elevated glucose with highest value at 24 hour-post stress, then declined till at 72 hrs. This pattern of glucose concentration might be attributed to the non specific heat stress response which lead to secretion of glucocorticoids with increased fuel demand to escape of hot water. The irregular pattern of glucose level is coincided with (Suarez and Mommsen, 1988 and Iwama, et al;1999), in addition to the correlation between the behavioral measurements and the cortisol concentration as reported by (Sloman. et al;2001). Moreover, variable values

variety of aquatic habitats (Wendelaar Bonga,1997).

3) Heterophil/Lymphocyte (H/L) ratios post heat stress

Table no (5) revealed the heterophil/Lymphocyte ratio as an indirect measure of stress increased in values of H/L in comparison to the control this might be due to the challenging with *A. hydrophila* pathogenic strain which represented cumulative response of multiple stresses, the result confirmed the impact of higher critical water temperature on Nile tilapia internal homeostasis and the ratios of blood WBCs (mainly H/L).

Table (4): Effect of warm-water stress on Nile tilapia plasma glucose ,cortisols and total plasma proteins.

Parameter	Post 1 hr.	Post 24 hr.	Post 48 hr.	Post 72 hr.
Time				
Glucose mg/dl	142.77	191.57 ±	81.63	64.46
Cortisols µg/dl	30	30	24.2	20
T.P.P. g/dl	0.5	0.5	0.5	0.5

4) Histopathological Findings

The Histopathological finding of stressed Nile tilapia is shown in plate no.2. The gills showed congestion of lamellar and branchial blood vessels associated with large areas of hemorrhage. There was lamellar telangiectasis in primary and secondary lamellae accompanied with focal inflammatory cells aggregates. (micrograph 7,8). The liver section remarked by congestion of central vein and sinusoids accompanied with areas of hemorrhage. (micrograph 9) It worth mention that spleen sections showed no clear pathological lesions. The intestinal section expressed severe sub-mucosal congestion and hyperplasia in the mucus secreting cells and sub-mucosal hemorrhages (micrograph 10). the brain revealed focal

Table (5): Heterophil/Lymphocyte (H/L) ratios post heat and challenge stress.

Time	Heterophil no.	Lymphocyte no.	H/L ratio
1hr	60	36	166.67
24	52	48	108.33
48hr (challenge)*	68	32	212.5
72hr	56	44	127.27
Control	20	89	25.00

* challenged by I/P injection of 0.2 ml of 24 hr broth culture of *A. hydrophila* containing approximately 10^6 CFU/ml.

area of hemorrhages (micrograph 11) The mentioned lesions in different organs represented signs of acute inflammatory response as reported by (Robert ,2001) who mentioned that ,the inflammatory response is the basic response to tissue damage whatever caused by. The process of acute inflammation is initiated by capillary dilation, increase blood flow into area and increase lumen diameter of the lumen capillary fenestrae which allow the largest serum protein molecules to exude into the tissue resulting in edema.

5) The challenge response

The results of challenged Nile tilapia by *A.hydrophila* is shown in table (6). The clinical picture of the heat stressed Nile tilapia post challenge revealed serrated tail fin and darker skin color, Some fish showed severe congested gills and clear exophthalmia in both eyes. The PM changes of the challenged heat stressed Nile tilapia showed internal organs congestion.

Table (5): The results of challenged Nile tilapia by *A.hydrophila*

Groups	Fish mortalities /hour							Total death %
	24 hr	48 hr	72 hr	96 hr	120	144	168	
G.1	8	2	---	---	---	---	---	66.6%
Total no	15							
G.3	1	5	---	---	---	---	---	
Total no	15							40%

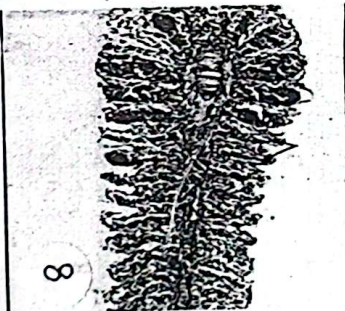
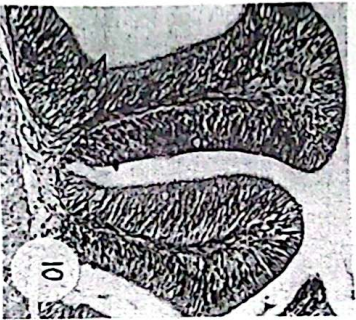
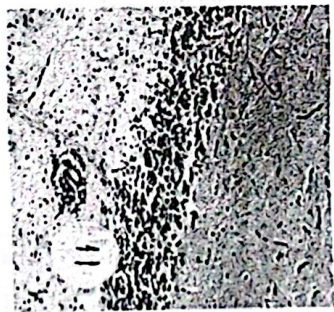
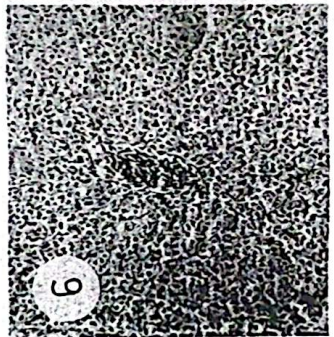
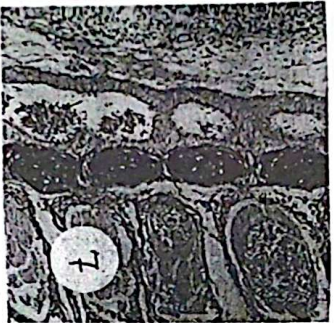
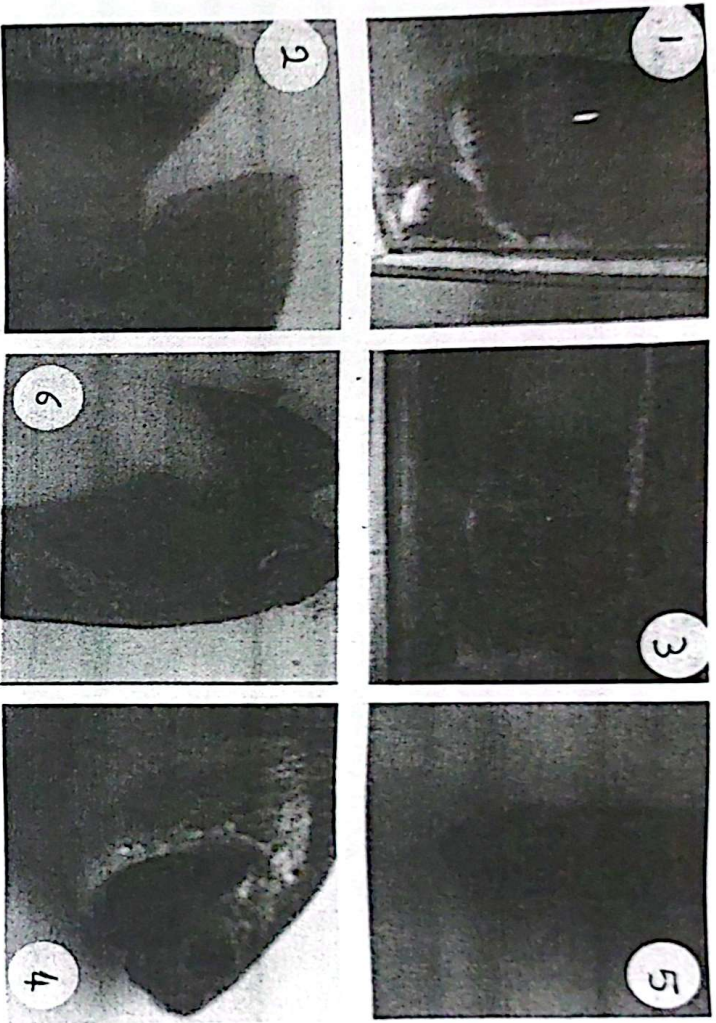


Plate (2)

An important tool for measuring the health status of the fish is diseases challenge. This technique provides an opportunity to determine if the xenobiotics exposure can alter the ability of fish to respond immunologically to bacteria known to be harmful or pathogenic to the species in its environment (Arkoosh, et al; 2005). On 48hr post heat exposure fish were challenged by *A. hydrophila* pathogenic strain, they were totally morbid (100% morbidity). The mortality 10/15 (66.6%) for challenged-heat stressed fish in comparison to control challenged 6/15 (40%). The variation in the results may be due to the predisposing effect of physical environmental stress (the high temperature) to attract infection with *A. hydrophila* bacteria and increase mortality. The result coincided with that recorded by (Roberts & Sommerville, 1982; Lio-po et al; 1983; Faisal, et al; 1989; Sisti et al; 1998 ; Plumb, 1999 and Wood and Bruno, 1999).

The clinical picture of the *A. hydrophila* infected fish showed severe tail and fin rot and darker skin color, Some fish showed severe congested gills and clear exophthalmia in both eyes together with hemorrhagic strikes on the skin with erosions and area of detached scales and slight ascitis. The PM changes of the challenged heat stressed Nile tilapia showing internal organs congestion. The finding is coincided by (Aoki, 1999) who stated that the clinical picture of infected Nile tilapia by *A. hydrophila* was manifested by cutaneous haemorrhage of the fins and trunk, this

is attributed by the fact that *A. hydrophila* is toxin producer thus induce toxemia and affect the capillaries in the sub cutaneous layer of the dermis, in addition to, the other clinical signs and PM lesions.

From the present work it was concluded that there was a negative impacts from heat stress environmental pollution resulted in the increased susceptibility of Nile tilapia to *Aeromonas hydrophila*. Strict measure must be directed to pan the alterations of temperatures in the fish ecosystem by correct transportation and gradual acclimation of fish in its new habitats.

Plate no (1):

Fig 1: Heat stressed Nile tilapia escaped toward glass wall of aquarium away of the hot point, some were recumbent at bottom (photo from inside water)..

Fig 2: Heat stressed Nile tilapia showed serrated tail fin and darkness of the skin.

Fig 3: Heat stressed Nile tilapia aggregating toward the source of oxygen (aerator). (photo from inside water).

Fig 4: Heat stressed Nile tilapia revealed severe congested gills.

Fig-5: Heat stressed Nile tilapia showing dark skin coloration and slight exophthalmia in both eyes.

Fig 6: PM of heat stressed Nile tilapia showing internal organ congestion.

Plate (2)

Micrograph 7,8 : The gill of heat stressed Nile tilapia showed congestion of lamellar and branchial blood vessels associated with large areas of hemorrhage. There was lamellar telangiectasis in primary and secondary lamellae accompanied with focal inflammatory cells aggregates.

Micrograph 9: The liver of heat stressed Nile tilapia remarked by congestion of central vein and sinusoids accompanied with areas of hemorrhage.

Micrograph 10: The intestinal section of heat stressed Nile tilapia expressed severe sub-mucosal congestion, hyperplasia in the mucus secreting cells and sub-mucosal hemorrhages

Micrograph 11: The brain of heat stressed Nile tilapia revealed focal area of hemorrhages.

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