Asian Fisheries Science **16** (2003): 269-278
ISSN: 0116-6514
https://doi.org/10.33997/j.afs.2003.16.3.010

Asian Fisheries Society, Manila, Philippines

The Effects of Stressful Conditions on an Artificial *Aeromonas hydrophilia* Challenge in Hybrid Catfish (*Clarias gariepinus* x *Clarias batrachus*)

M. CRUMLISH[1], T. SOMSIRI[2] and KD. THOMPSON[1]

[1]*Institute of Aquaculture*
Stirling University
Stirling, FK9 4LA Scotland
UK

[2]*Aquatic Animal Health Research Institute*
Kasetsart University
Bangkok
Thailand

Abstract

Catfish (*Clarias* sp.), cultured extensively and intensively throughout Southeast Asia, are generally regarded as robust animals, which do not readily succumb to stress-associated mortalities. However, they frequently encounter common environmental stimuli on the Thai fish farms, which are known to result in a stress response in aquatic animals. This in turn can compromise their immune defences against disease. The laboratory-based study discussed here examined the effect of various environmental stressors on the disease status of the animal when artificially bath challenged with *Aeromonas hydrophila*.

The highest percentage of total mortalities was found in the group receiving high temperature stress alone compared with the other stress-only groups. The highest percentage mortality was experienced in the fish group receiving low water temperature and a bacterial bath challenge. Control fish also died, but these deaths were thought to be due to wounds inflicted during fighting with fish held in the same tank.

Introduction

Cultured fish are unavoidably exposed to wide ranges of stimuli associated with environmental and husbandry challenges (Schreck 1996), where the physiological response to a stressor is similar in both teleosts and higher vertebrate species (Wedemeyer, Barton and McLeay 1990). Stress responses provide the animal with an ability to cope in the short-term during exposure to the encounter and increase its chance of survival under adverse conditions (Pickering 1997). The three separate stages of a stress response described by Seyle (1973) were alarm, resistance and exhaustion. The resulting response produced by the fish depends on the severity and the duration of exposure to the stimulus involved (Wedemyer 1996). Cognitive stimuli produce a stress response in the animal via neural and endocrine systems, with subsequent secretion of a wide range of hormones and factors used to control the response. Immunomodulation is often associated with a stress response in exposed fish (Angelidis, Baudinlaurencin and Youinou 1987; Möck and Peters 1990; Thompson, White, Fletcher, Houlihan and Secombes 1993; Sunyer, Gomez, Navarro, Quesada and Tort 1995; Yin, Lam and Sin 1995; Tort, Sunyer, Gomez and Molinero 1996), leaving them immunocompromised and susceptible to disease (Fries 1986).

All fish species have optimal environmental requirements, and when they are cultured under these conditions maximum growth rates are achieved with reduced disease susceptibility (Tucker and Robinson 1990). Animals are able to modify their behaviour in response to sub-optimal conditions, but if the stress response becomes maladpative, the fish can die. Tolerance to stress varies between fish species, but also depends on other factors such as age, sexual maturity, and the general health status of the animal (Tucker and Robinson 1990).

Farmed catfish frequently encounter and tolerate poor environmental conditions, which are well below the considered optimal. In channel catfish (*Ictalurus punctatus*) farms in America, episodes of low dissolved oxygen levels are one of the most frequent water quality problems encountered and this, in turn, can result in increased numbers of stress-associated mortalities (Tucker and Robinson 1990). Supplying an optimal and stable environmental temperature is important, as fish are poikilothermic with their internal temperature being reflected by the external environment. Bly and Clem (1991) demonstrated the importance of temperature on the immune response of channel catfish when fish were subjected to a 24-h low temperature shock, after which a significant reduction in lymphocyte responses occurred and this lasted for 3 to 5 weeks after the event.

High stocking densities can often be stressful for farmed fish populations. This is due not only to the decrease in the amount of available oxygen in the water but also as a result of an increase in the production of toxic substances in the water. The amount of suspended solids in the water is important as high levels of suspended solids (both organic and inorganic) can evoke a stress response, which can be species-specific. As Redding, Schreck and Everest (1987) found increased plasma cortisol levels in coho (*Oncorhynchus mykiss*) and steelhead salmon (*Salmo gairdneri*) when exposed to high (2 to 3 g/L) and low (0.4 to 0.6 g/L) levels of suspended solids. Channel catfish, however, can tolerate very high levels of suspended solids, which would result in high mortalities in other fish species. Fingerling channel catfish have been reported to survive in water with 100,000 ppm suspended solids, although behavioural changes were noted at 20,000 ppm (Wallen 1951 cited in Schreck et al. 1987).

The aim of the present study was to measure the effects of a variety of environmental conditions (stressors) often observed during the production of hybrid catfish cultured in freshwater earthen-ponds in Thailand on disease susceptibility when fish were artificially bath challenged with an opportunistic bacterial pathogen.

Materials and Methods

Fish

Hybrid catfish (*Clarias gariepinus* x *C. batrachus*), purchased from a farm outside Bangkok, Thailand, were held and acclimated in the aquarium facility at the Aquatic Animal Health Research Institute, Bangkok for one week. Fish, approximately 15g in weight, were fed daily on commercial pellets and given 70% water changes every 2 days. The water temperature during this time was $28^{\circ} \pm 3^{\circ}$C. The fish were checked for any overt signs of disease, which included behavioural abnormalities.

Bacteria

The challenge organism, *Aeromonas hydrophila* (identified as T_4), was obtained from the bacterial collection at the Institute of Aquaculture, University of Stirling. Bacteria were cultured on tryptone soya agar (TSA) (Difco, UK) and their identity confirmed by primary (Gram stain, shape, motility, oxidase, oxidation/fermentation) and secondary biochemical tests (API 20E BioMeriuex, France) following the methods of Frerichs and Millar (1993). A bacterial suspension in tryptone soya broth (TSB) (Difco) was grown to log-phase at 28°C for 24-h, centrifuged at 2000 x g for 15 mins and washed twice with sterile physiolgical saline. To enhance virulence, a bacterial suspension at a concentration of 1 x10^8 colony forming units (cfu) ml^{-1} obtained from a standard curve of cfu versus absorbance at 610 nm was passaged twice by intraperitoneal injection in adult fish and pure growth was recovered from the liver of dead animals. Bacteria were prepared at 1 x10^5 cfu ml^{-1} for the challenge experiments and the concentration was verified using the Miles and Misra colony count method (Heritage, Evans and Killington 1996).

Experimental protocol

The surface of each animal including the controls was scored (0.5 cm in length) with a sterile needle, sufficient to remove skin but not to draw blood. There were 6 experimental (stressful) conditions tested and each experiment consisted of four groups: (1) a control group (which received no bacterial challenge or potential stress); (2) bacterial challenge only; (3) stress only and (4) a combination of bacterial challenge and stress (Table 1). A total of 100 fish were used per experimental treatment and these were randomly separated into five fish per tank with five replicates per experimental group so each group, except the high stocking density fish groups had a total of 25 fish. All animals were placed into glass tanks containing 20 L of freshwater at 28°C.

Experimental treatments

Animals in groups A and B (Table 1) were placed into tanks containing water at the desired temperatures for 1 h and after bacterial challenge they were then maintained in water at 28°C for the remainder of the experiment. The temperature-related experiments were carried out in a temperature-controlled aquarium. Fish held in group E were kept in plastic bags with a small volume of water for a 1-h journey. Twice the number of fish per tank (10 instead of 5 fish per tank) compared with the control fish were used for experimental treatment C. Animals in group F were removed from the water and kept in a bucket for 2 h before being placed back into the tanks. No control fish group was included for experiments C or F. After encountering the stressor, fish from the appropriate groups were challenged by bath with *A. hydrophila* at 1×10^5 cfu ml^{-1} for 1 h. After this time the water was changed. Twenty-four h post-stress and bacterial challenge, all animals were fed daily and water changed every two days, except for fish in the poor water quality trial, which received no water changes. Fish were monitored twice daily for seven days, mortalities recorded and bacterial swabs prepared

Table 1. Experimental Treatments and Groups

	Treatment Groups	Experimental Groups
A	High water temperature (35°C)	A 1 Control A 2 Bacteria A 3 Stress A 4 Combined
B	Low water temperature (20°C)	B 1 Control B 2 Bacteria B 3 Stress B 4 Combined
C	High stocking density	C 2 Bacteria C 3 Stress C 4 Combined
D	No Water Exchange	D 1 Control D 2 Bacteria D 3 Stress D 4 Combined
E	Transportation stress	E 1 Control E 2 Bacteria E 3 Stress E 4 Combined
F	Removed from water	F 2 Bacteria F 3 Stress F 4 Combined

from the livers of any dead fish. Mortalities due to *A. hydrophila* were confirmed as described above.

Statistical analysis

The data was analysed using a Chi-Square Test, where $P \leq 0.05$ was considered statistically significant.

Results

Fish mortalities were recorded for all of the experimental groups in all of the 6 treatments. The total mean percentage mortalities were found to differ between the various experimental groups (Table 2). Most of the mortalities occurred in the first 1 to 3 days of the experimental treatments, however, some deaths continued to occur in some of the tanks at the end of the trial on day 7. The highest percentage of total mortalities (84%) was found in treatment group B (low water temperature) in the combined group compared with any other treatment or experimental group (Table 2). The total percentage mortalities were high in the combined experimental groups for all of the treatments (Table 2), however, high percentage of fish deaths were also recorded in the treatment groups receiving bacterial challenge only (Table 2). Except in treatment group A and F, the percentage mortalities were greater for all treatment groups in the bacteria only groups compared with the stress only groups (Table 2). Statistically significant differences were found between the control and experimental groups for treatments A, B, D and E (Table 3), however, no control groups were included in treatment C and F. No statistically significant differences were detected in experimental groups for treatments A, B and C (Table 3).

Bacteria recovered from the liver of dead fish were all confirmed as *A. hydrophila*, and was the only bacterial species recovered from challenged fish. Some control animals also died, due to wounds inflected as a result of fighting. Only non-specific growth was recovered from the livers of the dead control fish and was not identified further.

Table 2. The total percentage (%) mortalities of fish per treatment group exposed to various experimental conditions

Treatment	Total Mortalities (%)			
	Control	Bacteria Only	Stress Only	Combined*
High Temperature (35^0C)	24	52	64	64
Low Temperature (20^0C)	16	68	20	84
High Stocking Density	n/a	52	42	76
No water change	32	76	32	80
Transportation	4	28	4	24
Removed from water	n/a	4	20	36

*stress and bacterial challenge
n/a = data not available

Discussion

The impact of physical, environmental and biological stress on increased disease susceptibility in cultured cannel catfish has been clearly demonstrated (Plumb 1984, Ellsaesser and Clem 1986). Ciembor, Blazer, Dawe and Shotts (1995) demonstrated a higher rate of enteric septicaemic infection in fish exposed to *Edwardsiella ictaluri* by either intraperitoneal injection or bath challenge when subjected to stress than without stress. In American channel catfish farms outbreaks of motile aeromonad septicaemia due to the potential opportunistic pathogen *A. hydrophila* are experienced, particularly when fish are exposed to both physical and environmental stressors (Plumb 1994).

It has been shown that a stress response in fish results in the production of the hormone cortisol, which has an immunosuppressive effect on both the innate and the adaptive immune response of the animal (Schreck 1996). In the study presented here, plasma cortisol levels were not measured and this should be included in further studies to demonstrate a true stress response in the exposed animals. The total percentage mortality was expressed in the presented study as an indicator of the effect of different types of environmental stimuli which farmed catfish in Thailand may encounter throughout a production cycle. The effects of the different stessors on the total mortality varied considerably but were reflective of the low level mortalities experience by small-scale Thai catfish farmers (pers.com.).

Table 3. Statistical analyses of total mortalities per treatment group

Treatment group	P Value	χ^2 Value	Comment
A. High Temperature (35°C)	0.014	χ^2_3 = 10.684	Highly significant increase in mortality between the control and experimental groups
	0.607	χ^2_2 = 1	No significant difference between bacteria, stress and combined group
B. Low Temperature (20°C)	< 0.000	χ^2_3 = 35.126	Highly significant increase in mortality between all experi mental and control groups
	0.185	χ^2_1 = 1.754	No significant difference in the percentage mortality between the bacteria and combined group
C. High stocking density	0.059	χ^2_2 = 5.669	No significant difference between treated groups
D. No water change	< 0.000	χ^2_3 = 21.455	Highly significant difference between the controls and treated groups
E. Transportation	0.022	χ^2_3 = 9647	Significant increase in mortality between control and treated groups
F. Removed from Water	0.018	χ^2_2= 8	Significant increase in mortality found between the bacteria and combined groups

χ^2 = Chi squared value, P = probability

Except in the fish group receiving transportation stress, the highest percentage mortalities were recorded in the experimental groups receiving both stress and bacterial challenge compared with the stress only, bacterial challenge only or control fish. The percentage mortalities in the stress only group were highest in the fish given a high water temperature and lowest in the groups receiving transportation stress. It was interesting to note, however, that in the bacterial challenge groups alone, the highest percentage mortality was found in the tanks receiving no water exchange and fish given a low temperature (20°C) stressor. It may be that the levels of bacteria in the water increased as a result of no water exchange and this resulted in an increase in the number of bacteria present in the surrounding environment, hence an increase in the potential interaction between host and bacteria present in the water. To confirm this, viable bacterial numbers in the water should have been monitored daily.

The higher level of mortalities that occurred when the fish were kept at 20°C may have been due to immunosuppression of the animals rather than an affect of the bacterial level present in the water, thus making the fish more susceptible to disease from the bacterial challenge. Although no measurements of hybrid catfish immune response was determined in the study presented, Le Morvan, Clerton, Deschaux and Troutaud (1997) reported that reduced temperature adversely affected the teleost immune response. However, the phagocytic cells of channel catfish appeared to be more resistant to low temperature compared with lymphocytes (Ainsworth, Dexaing, Waterstrat and Greenway 1991). Le Morvan et al. (1997), found that exposure to low environmental temperature enhanced phagocytic activity of carp (*Cyprinus carpio*) macrophages when assayed at ambient temperature. Temperature-mediated affects on the cellular immune response of hybrid catfish have not yet been reported.

Although catfish are regarded as a robust fish species with high tolerance levels, they still appeared to succumb to transportation-induced stress. In channel catfish culture, mortalities due to transportation occur because large numbers of fish are transported in a small volume of water (Tucker and Robinson 1990). The transportation of live catfish demands a high level of care and attention if the fish are to arrive in a good condition, ready for the market. In this study, fish receiving transportation as a stressor with and without bacterial challenge had the lowest percent mortality compared with the other experimental groups but mortalities still occurred. One of the main problems in transporting fish stock is maintaining a high level of dissolved oxygen (Wedemeyer 1996). Catfish, as air-breathers, are frequently transported from the Thai farms to local markets in very low water levels and without additional oxygen. The results from the transportation treatment study may suggest that transportation may not be such a severe stressor for hybrid catfish as they are able to tolerate relatively low oxygen levels. Fish in the present experiment were transported in an air-conditioned van for a relatively short period of time, and this may have reduced the impact of the transportation stress, by cooling the animals, and hence inhibiting movement and conserving energy to help the fish cope with the stress.

This may be further supported by the finding that the fish group receiving low temperature stress only had the lowest percentage mortaility.

Throughout the experimental period, it was observed that some of the control group fish also died. The percentage mortality was highest in the no water exchange control group. Strong spines have developed on the catfish pectoral fins to provide locomotion and a protective function. They are not poisonous but help these fish to move when migrating across land (Viveen, Richter, van Oordt, Janssen and Hulsman 1977). These fins can often cause skin damage on animals sharing the same tank space, as hybrid catfish do not have any form of protective scales. In some cases it was observed that the control fish had severe injuries to their body surface, which may have caused the animals to become physically compromised resulting in mortalities in the control fish groups. Injuries sustained from fighting may also have facilitated microbial entry into the fish. When the control fish were sampled, only non-specific bacterial growth was recovered from the liver. The mixed bacterial colonies recovered from the control fish were not identified to species level.

Although this was a laboratory-based experiment the fish were from a farmed population, and the potential stressors applied were based on actual environmental conditions commonly observed on the Thai catfish farms. Stress-related diseases do occur on the catfish farms in Thailand, as poor water quality can occur due to inappropriate drainage, resulting in high levels or organic suspended solids. Also, during the wet season or in the winter, the daily water temperatures can fluctuate overnight, resulting in farmed stock that are increasingly susceptible to disease by opportunistic pathogens (Tonguthai, Chinabut, Limsuwan, Somsiri, Chanratchakool, Kanchanakhan and MacRae 1993). The data from this experiment would suggest that catfish although robust in comparison with other cultured teleosts do succumb to stress and that this may exacerbate the onset of bacterial diseases when challenged experimentally. The bacterial challenge levels applied were taken from the total viable bacterial counts previously found on catfish farms and so were thought to accurately reflect farming situations in Thailand (unpublished data). However, only a single bacterial species was given to the fish and on the farms various potential opportunistic freshwater bacterial species can be found rather than a single bacterial species (unpublished data).

It was found that farmed catfish could withstand the different stressors to varying degrees as shown by the percentage mortalities. However, these were only applied once singularly and the cumulative effect of the stress or effect of different stressors given at the same time on farmed hybrid catfish is unknown. It would be very rare if a single stressor was encountered only once throughout the production cycle. As temperature changes, fluctuating dissolved oxygen levels, transportation, handling and grading, social interactions as well as the presence of pathogenic microbes in the water are all potential stressors that occur in rearing conditions (Wedemeyer 1996). Bacterial entry may also be facilitated by injuries as catfish do not have protective scales and their sharp fins can easily tear the skin of another fish within the immediate vacinity.

Further studies are required to measure the impact of single and cumulative stress on hybrid catfish held under farming conditions and to investigate the relationship between these and disease susceptibility from opportunistic and specific pathogens. In the study presented here, only the percentage mortalities were measured to detect the effect of stress on bacterial challenge. However, future studies should also measure behavioural (e.g inappetance, fighting) and physiological parameters (e.g plasma cortisol or glucose levels) (Iwama, Morgan and Barton 1995), to accurately detect the extent of the stress response produced. Similarly, further work is required to understand the effect of single and cumulative stress on the immune response in farmed hybrid catfish and to determine the effect on stress-induced mortalities due to disease.

References

Ainsworth, A.J., C. Dexaing, P.R. Waterstrat and T. Greenway. (1991) Effect of temperature on the immune system of Channel catfish (*Ictalurus punctatus*). 1. Leukocyte distribution and phagocyte function in the anterior kidney at 10°C. Comparative Biochemistry and Physiology A 100 (4): 907-912.

Angelidis, P., F. Baudinlaurencin and P. Youinou. (1987) Stress in rainbow trout, *Salmo gairdneri* effects upon phagocyte chemiluminescence, circulating leukocytes and susceptibility to *Aeromonas salmonicida*. Journal of Fish Biology 31 (Supplement A): 113-122.

Bly, J.E. and L.W. Clem. (1991) Temperature mediated processes in teleosts immunity: In vitro immunosuppression indiced by in vivo low temperatures in channel catfish. Veterinary Immunology and Immunopathology 28: 365-377.

Ciembor, P.G., V.S. Blazer, D. Daine and E.B. Shotts. (1995) Susceptibility of channel catfish to infection with *Edwardsiella ictaluri*: Effect of exposure method. Journal of Aquatic Animal Health 1 (2): 132-140.

Ellaesser, C.F. and L.W. Clem. (1986) Haematological and immunological changes in channel catfish stressed by handling and transport. Journal of Fish Biology 28 (3): 511-521.

Frerichs, G.N. and S.D. Millar. (1993) Mannual for the Isolation and Identification of Fish Bacterial Pathogens. Pisces Press, Stirling, Scotland. 58 pp.

Fries, C.R. (1986) Effects of Environmental Stressors and Immunosuppressants on Immunity in *Fundus heteroclitus*. American Zoologist 26: 271-282.

Heritage, J., E.G.V. Evans and R.A. Killington. (1996) Introductory Microbiology pp 106-108. Cambridge University Press.

Iwama, G.K., J.D. Morgan and B.A Barton. (1995) Simple field methods for monitoring stress and general condition of fish. Aquaculture Research 26: 273-282.

Le Morvan, C., P. Clerton, P. Deschaux and D. Troutaud. (1997) Effects of environmental temperature on macrophage activities in carp. Fish & Shellfish Immunology 7: 209-212.

Maule, A.G., R.A. Tripp, S.L. Kaattari and C.B. Schreck. (1989) Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus tshawytscha*). Journal of Endocrinology 120: 135-142.

Möck, A. and G. Peters. (1990) Lysozyme activity in rainbow trout, *Oncorhynchus mykiss* (Walbaum), stressed by handling, transport and water pollution. Journal of Fish Biology 37 (6): 873-885.

Pickering, A.D. (1997). Husbandry and Stress. In: Furunculosis. Multidisciplinary Fish Disease Research: 178-202. Eds: Bernoth, E-M., Ellis, A.E., Midtlng, P.J., Oliver, G. and Smith, P. Academic Press.

Plumb, J.A. (1984) Relationship of water quality and infectious diseases in cultured channel catfish. In:Fish Pathogens and Environment in European Polyculture. Ed:Olah, J. 23: 189-199.

Redding, J.M., C.B. Schreck and F.H. Everest. (1987) Physiological effects on coho salmon and steelhead of exposure to suspended solids. Trans. American Fish, Soc. 116: 737-744.

Schreck, C.B. (1996) Immunomodulation: Endogenous Factors. In: The fish immune systems: Organism, Pathogen and Environment pp 311-337. Eds: Iwama, G and Nakanishi, T. Academic Press.

Seyle, H. (1973) The evolution of the stress concept. American Scientist 61: 692-699.

Sunyer, J.O., E. Gomez, V. Navarro, J. Quesada and L. Tort. (1995) Physiological responses and depression of humoral components of the immune system in gilthead seas bream (*Sparus aurata*) following daily acute stress. Canadian Journal of Fisheries and Aquatic Science 52 (11): 2339-2346.

Thompson, I., A. White, T.C. Fletcher, D.F. Houlihan and C.J. Secombes. (1993) The effect of stress on the immune response of Atlantic salmon (*Salmo salar* L.) fed diets containing different amounts of vitamin C. Aquaculture 114:1-18.

Tonguthai, K., S. Chinabut, C. Limsuwan, T. Somsiri, P. Chanratchakool, S. Kanchanakhan and I. MacRae. (1993) Handbook of Hybrid Catfish: Husbandry and Health. pp37. The Aquatic Animal research Institute, Department of Fisheries, Kasetsart University, Bangkok, Thailand.

Tort, L., J.O. Sunyer, E. Gomez and A. Molinero. (1996) Crowding stress induces changes in serum haemolytic and agglutinating activity in the gilthead sea bream *Sparus aurta*. Veterimary Immunology and Immunopathology 51 (1-2): 179-188.

Tucker, C.S. and E.H. Robinson. (1990) Channel Catfish Farming Handbook. Van Nostrand Reinhold, New York. pp 454.

Viveen, W.J.A.R., C.J.J. Richter, P.G.W.J. van Oordt, J.A.L. Janssen and E.A. Hulsman. (1977). Practical Manual for culture of the African walking catfish (*Clarias gariepinus*), p7.

Wedemeyer, G.A. (1996) Physiology of fish in intensive culture systems. Published by Chapman & Hall, USA pp.226.

Wedemeyer, G.A., B.A. Barton and D.J. McLeay. (1990). Stress and acclimation. In:Methods for Fish Biology. (Eds: Schreck, C. and Moyle, P). American Fisheries Society, Bethesda, Maryland: 451-489.

Yin, Z., T.J. Lam and Y.M. Sin. (1995) The effects of crowding stress on the nonspecific immune response in fancy carp (*Cyprinus carpio* L.). Fish & Shellfish Immunology 5: 519-529.

Manuscript received 4 October 2000 ; Accepted 24 June 2001