RECENT ADVANCES IN STUDIES ON ACUTE DISEASES OF FISHES

—A REVIEW

Radhanath Pal

Bulletin No. 35
DECEMBER, 1982

CENTRAL INLAND FISHERIES RESEARCH INSTITUTE
BARRACKPORE-743101. WEST BENGAL
INDIA

FOREWORD

Fish disease and its control, pond hygiene and prophylactic measures are some of the aspects of vital importance in fish husbandry. A correct understanding of the problem of fish diseases and their control has become all the more important as we embark upon intensive fish culture in traditional systems as well as in some specialized systems like cage-culture, sewage-fed fish culture, etc., It is well known that fish health is inextricably associated with environment, and environmental stress in many cases has rendered the fish vulnerable to fish diseases. These aspects as well as bacteria, virus and parasites-caused disease of fishes are examined in this review in some detail for the benefit of research workers. It is also hoped that the manual would be of use to fish farm scientists, managers and entrepreneurs interested in culture and maintenance of healthy fish stocks.

Dr. A. V. Natarajan
Director

Credits

Edited by : M. J. Bhagat

Associates : A. R. Chowdhury

Printed by : Roman Printers
37 Andul Road Howrah 711 109

Published by : Dr. A. V. Natarajan,
Director,
Central Inland Fisheries Research Institute
Barrackpore-743 101, West Bengal, (India).

CONTENTS

.....Contd.

Contents

CHAPTER 1

Introduction

Captured fishes from natural resources seldom exhibit any disease manifestation, apart in exceptional cases. They may often harbour a few parasites but such an infection less frequently causes much harmful effects on their hosts. As such, captured fishes from natural waters generally look healthy. But when fishes are cultured in smaller bodies of water, they often get diseased for which various reasons can be assigned. It may be mentioned here that terrestrial animals live in a better condition than fresh water fishes, which have to adjust with the constant changes in their environment. Such changes are often caused by temperature, light, dissolved gases, water chemistry, pollution, micro and macro-organisms, etc. In smaller bodies of water, fishes also undergo other changes due to population density, diet composition, excess of nitrogenous and other metabolites, stocking, hauling, handling and disease treatments. As a result, the stocking material suffer from non-maintenance of homeostatis (Wedemeyer, Meyer and Smith, 1976).

Homeostatis

In the last century Claude Bernard first coined this term—"homeostatis", which meant maintenance of physiological condition within narrow limits of an organism (Nelson, Robinson and Boolootain, 1967).

Fish being a poikilothermal animal has to acclimate much with its environment, which is very much dynamic. As such, the external environment of a fish has a great influence on its internal environment. Thus, there is always a possibility of causing "stress" on the maintenance of homeostatis of a fish by its external environment ; such stress if continued for long the fish may pose disease problems.

Stress

Originally "stress" was defined by Seyle (1950) as "The sum of the physiological response by which an animal tries to maintain or reestablish a normal metabolism in the face of a physical or chemical force". Brett (1958) correlated "stress" with the fish

disease situation, *i. e.* when the normal functioning is significantly reduced and finally may manifest in death.

Primary effects of stress on fishes

Carps suffering from primary stress exhibit increased level of circulating corticosteroids (Chavin and Singley, 1972 ; Redgate, 1974 ; Spieler, 1974 ; Fryer, 1975 and Singley and Chavin, 1975). Teleosts suffering from depletion of oxygen show increases in their plasma as the primary effects of stress (Nanko and Tomilson, 1967 ; Mezeaud, 1971).

Secondary effects of a stress on fishes

Secondary effects of stress are manifested in many physiological changes, some of them are mentioned below (Mezeaud, Mozeaud and Donaldson, 1977) :

—Increased blood pressure and heart rate ;
—Increased level of blood sugar and lactate ;
—Increased in the number of thrombocytes ;
—Increased level of liver glycogen ;
—Decreased in the number of white blood corpuscles ;
—Decreased level of serum protein and blood chloride ;
—Decreased inflammatory response ; and
—Immuno suppression and changes in mucus production.

According to Seyle (1973) a series of morphological, bio-chemical and physiological changes are manifested in higher verterbrates when they are subjected to stress. Such changes are collectively called as general adaptive syndrome (G. A. S.).

General adaptive syndrome

GAS can be defined as the cumulative effects of environmental stress on higher vertebrates. The changes occuring in a stressed animal have the following three phases :

1. The alarm reaction ;

2. The stage of resistance when the animal tries to adapt with its environment, the maintenance of its homeostatis ; and
3. The stage of exhaustion when adaptation has failed and the animal has lost homeostatis. In this connexion it can be mentioned that homeostatis of an animal is controlled by its endocrine and nervous systems. Seyle (1950) has differentiated the functions of endocrine and nervous systems of a stressed animal as follows :

   —ACTH (Adrenocorticotropic hormone) is released from pituitary after receiving the neurotropic impulse through the hypothalamus. ACTH stimulatory hormones are cortisone, corticosterone, and epinephrine; and

   —Sympathetic nervous system is hypertensioned. As a result, (a) spleen contracts and additional erythrocytes enter the circulatory system, and (b) both respiration rate and cardiac output increase, and systolic blood pressure also rises.

ACTH release, controlled by hypothalamus in teleost, has also been recognised by Hill and Henderson (1968). Many workers have also demonstrated the secretion of stress hormones in salmonids (Black *et al.*, 1961 ; Hans *et al.*, 1966 ; Donaldson and Mc Bride, 1967 ; Fagerlund, 1967 ; Nakano and Tomilson, 1967 ; Hill and Fromm, 1968 ; Wedemeyer, 1969).

Clinical methods for the assessment of the effects of environmental stress on fish health have been documented by Wedemeyer and Yasutake (1977).

From the above statement it is evident that for disease manifestation of cultured fishes, an interrelation among host, pathogen and their environment is always in existence.

Relationship among host, pathogen and their environment

In a body of water fishes and their pathogens may be encounted without any disease manifestation of the former in general. This triad relationship was first considered by Meyer (1970) and Wedemeyer (1970), who postulated that stress is a very important factor in the outbreaks of infectious disease of fishes. Or, in other words, actually disease manifestation in fishes is a complex process where so many factors *viz.*, environmental conditions, host susceptibility, virulence of pathogen, are involved. As such, it

can never be considered as a simple interaction between the host and the parasite. Sometimes a fourth factory may also take part to cause the disease of a fish ; carp hemorrhagic septicemia may be taken for such an example ; poor sanitary condition of water tells upon fish health and causes host's susceptibility for infection vis-a-vis helps in rapid multiplication of the pathogen *Aeromonas liquefaciens*. Infection of *A. liquefaciens* is supposed to be a pre-disposing factor for the chronic virul infection (VHS) of the carp (Wedemeyer and Wood, 1974). For disease manifestation in fishes another aspect is also to be considered and that is Pathogenesis.

Pathogenesis

The genesis involved in "Pathos" (disease manifestation) of an organism can be termed as pathogenesis. A fish may suffer from disease due to either change in its environmental condition or infection of a pathogen. A generalised information has already been given as to how environmental stress acts upon disease manifestation. Pathogenesis due to viruses, bacteria, fungus, and invertebrates infections needs further clarification.

A successful pathogen, may potentially be infectious, must first find a susceptible host for its lodging and multiplication. This is not easy because the body of fish is covered with scales and there are indefinite number of epidermal cells which secrete mucus to get rid of a pathogen trying to get lodged on a susceptible fish. Water taken in through mouth is also thrown out by means of gills and opercular space. It is, thus, difficult for a fish pathogen to find its entry into its host. If it finds a way through the mouth, the pathogen is to survive in the acid and alkaline media of the alimentary tract. To tolerate the same is not easy for every pathogen. An easier way by which a pathogen gets entry in its host is a lesion on the skin or through other openings like mouth, eye, nostril, etc. As soon as the pathogen enters the circulatory system of its host, the former meets with various defence mechanism of the latter. Inflammation, Immune responses, etc., are such mechanisms (Anderson, 1974 ; Ritzkers 1980). However, getting an entry the pathogen must establish itself on the host so that it can multiply. A successful pathogen keeps its host alive for a longer period and thus causes a choronic disease of its host. But virulent pathogens cause acute disease to their hosts when the latter die within a short period.

Chronic and acute diseases

Chronic diseases of fishes are caused generally by less virulent bacteria, fungus,

protozoans, trematodes, cestodes, nematodes, annelids and crustaceans. Due to chronic disease mortalities within a fish population occur for a longer period when hardly 50% of the stocking material is lost altogether. But due to an acute disease the entire population is lost within a limited period. Due to oxygen depletion or pollution of lethal toxicants the entire fish stock may succumb within few hours. Virulent viruses or bacteria may cause acute disease of fishes too when the entire fish stock is lost within a period of 5-6 days. A lot of literature is already available on the chronic diseases of fishes and their remedial measures. As such, the present communique is restricted to the diseases of fishes due to environmental stress, viral, and bacterial infections. Attempts have also been made to supply information on preventive and remedial measures taken against such infectious diseases which generally break out in epidemic proportions. It will not be out of place to mention that purpose of the present communique is just to make the reader concious about the comment of Dr Sniessko (1972) —"... ... ... the most voluminous is the literature on fish parasites ... ... altogether 71% dealt with descriptions of parasites. Of the remaining 29%, 16% had the word 'disease' in the title, 7% were on ecology etc., 6% on control methods and 1·5% on Immunity". This is in sharp contrast to reports on diseases of fish caused by bacteria and virus where the study of the actiological agents received much less attention".

Literature cited

Anderson, D. P., 1974 — The fish's mechanisms for disease protection, *In* Fish Immunology. Ed. S. P. Sniessko and H. R. Axelrod T. F. H. Publishers. Neptune, N. J. : 54-95

Black, E. C., 1961 — Robertson, A. C. and Parker, R. R., Some aspects of carbohydrate metabolism in fish. *In* Comparative Physiology of carbohydrate metabolism in heterothermic animals. Ed. A. W. Martine, University of Washington Press, Seatle, Washington, 89-124

Brett, J. R., 1958 — Implications and assessment of environmental stress. *In* The investigation of fish power problems. H. R. MacMillan lectures. University of British Columbia : 69-97

Chavin, W. and 1972 — Singley, J. A., Adrenocorticosteroid of gold fish, *Carassius auratus. Comp. Biochem. Physiol.*, : 33 : 629-633

Donaldson, L. M. 1967 — and McBride, J. R., The effects of hypophysectomy in the rainbow trout, with special reference to pitutary internal sxis.

Gen. Comp. Endocrinol., : 9 : 93-101

Fagerlund, V. H. M. 1967 Plasma cortisal in relation to stress in adult sockeye salmon during the freshwater stage of their life cycle. *Gen. Comp. Endocrinol.* , **8** : 197-207

Fryer, J. N., 1975 Stress and adrenocorticosteroid dynamics in the gold fish, *Carssius auratus. Can. J. Zool.* , **53** : 1012-1020

Hans, S. O., 1966 Robertson, O. M , Wexler, B. C. and Krupp, M. R., Adrenocortical response to stress and ACTH in Pacific salmon (*Onchorhynohus ıshewytsche*) and steelhead trout at successive stages in the sexual cycle. *Endocrinol.* , **78** : 791-800

Hill, C. W. 1961 and Fromm, O. P., Response of the interrenal gland of rain bow trout (*Salmo gairdneri*) to stress. *Gen. Comp. Endocrinol.* , **11** : 69-77

Hill, J. J. and 1968 Henderson, N. E., The vascularisation of the hypothalmic-hypophyseal region of the eastern brook, *Salvelinus fontinalis. Am. J. Anat.*, **122** : 201-316

Meseaud, M. M. 1971 Recherches Sur la biosyntheses. Ph. D. Thesis es-Science. Uni versite de Paris : 130 pp (not seen in original)

Meseaud, M. M., 1977 Meseaud, P. and Donaldson, N. M., Primary and secondary effects of stress in fish. Some new data with a general review. *Trans. Am. Fish Soc.* , **106**(3) : 201-212

Meyer, F. P. 1970 Seasonal fluctuations in the incidence of disease in fish farms. Symposium on the Diseases of Fishes and Shellfishes. *Am. Fish Soc.*, Publn. No. **5** : 526 pp

Nakano, T. and 1967 Tomilson, N., Catecholamines and carbohydrate concentrations in rainbow trout in relation to physical disturbances. *J. Fish. Res. Bd. Can.* , **24** : 1701-1715

Nelson, G. E. 1969 Robinson, G. G. and Boolootian, R. A. 1., The interaction of control systems : Homeostatis. *In* Fundamental Concepts of Biology. A Willey International Edition, John Willey and Sons, Inc., New York : 143-154

Redgate, E. S. 1974 Natural control of pituitary adrenal activity in*Cyprinus carpio. Gen. Comp. Endocrinol.* , **22** : 33-41

Ritzkers, G. T., 1980 — Teunissen, A. G , Van O osterom, R. and Van Muiswinkel, W. B., The immune system of cyprinid fish. The immunosuppressive effect of the oxytetracycline in carp (*Cyprinus carpio* L.) : *Aquaculture* , **19**(2) : 177-89

Seyle, H., 1950 — Stress and the general adaptive syndrome (GAS). *Br. Med.J.*, **1** : 1383-92

— , 1973 — The evolution of stress concept. *Am. Sci.* ; **61** : 692-99

Singley, J. A. and 1975 — Chavin, W., Serum cortison in normal goldfish *(Carassius auratus* L.). *Comp. Biochem. Physiol.* , **504** : 77-82

Sniomzko, S. F., 1972 — Nutritional fish diseases. *In* Fish Nutrition. Ed. J. E. Halver ; Academic Press, New York & London : 401-437

Spioler, R. E., 1974 — Short term serum cortisal concentrations in goldfish subjected to serial sampling and restraint. *J. Fish, Res. Bd. Can.*, **31** : 1240-1242

Wedemeyer, G. A., 1969 — Stress induced ascorbate depletion and cortisal production in two salmonid fishes. *Comp. Biochem. Physiol.* , **29** : 1247-1251

— , 1970 — The role of stress in the disease resistance of fishes. *In* A Symposium on Diseases of Fishes and Shellfishes. Ed. S. F. Snieszko ; *Am. Fish. Soc.*, Publ. No. **5** : 30-35

Wedemeyer, G. A, 1976 — Meyer, F. P. and Smith. L., *In* Environmental Stress and Diseases of Fishes. Ed. S. F. Snieszko and H. F. Azelrod ; T. F. H. Publishers, Neptune, N. J. : 192 pp

Wedemeyer, G. A. and 1974 — Wood, J. W., Stress as a predisposing factor in fish diseases. *U. S. Fish Wildl. Serv.*, Fsh leafl. No. **38** : 1-7

Wedemeyer, G. A. and 1977 — Yasulake, W. T., Clinical methods for the assessment of the environmental stress on fish health. *U. S. Fish Wildl. Serv.*, Tech. Rep. No. **89**

CHAPTER II

Environmental stress and diseases of fishes

In the first chapter homeostatis and stress have been defined. The relationship among host (fish) pathogen and environmental condition has also been identified. However, the present chapter is to elaborate this relationship for a better understanding, because this is an important aspect of fish culture and the basis for making the enterprise a profitable one. Before the same is done, the physiology of freshwater fishes needs to be discussed along with their environmental condition. It is well known that every fish prefers an optimal condition for its growth and reproduction. Any alteration in the environmental condition causes 'stress' on the fish. If such an alteration increases arithmatically, the stress caused by the same on a community of fish will manifest in geometrical proportion which may reflect in the mortality of the entire community. As such, certain aspects of fish physiology in relation to environmental condition will be highlighted.

Fishes are poikilothermal animals and most of the freshwater fishes are amnolytic in habit. Accordingly, Na^+ and Cl^- ions are takenin by a freshwater fish and NH_4^+ and HCO_3^- are expelled out to maintain the ionic balance. Two enzymes *viz.*, deamination and carbonic anhydrase present in the gill epilthelia take active part in maintenance of the ionic balance. Blood flow in gills is also regulated by hormones *viz.*, adrenaline and acetylcholine (Steen and Kruysee, 1964). Acetylcholine regulates blood flow when oxygen demand for metabolic purposes is low. When fishes are stressed and demand for oxygen in more adrenaline (epinephrine) helps in increased blood fiow through the thin gill lamellae for facilitating maximum gas exchange rather oxygen uptake. For freshwater fishes the environment is hypo-osmotic ; as such, the kidneys of freshwater teleosts play a vital role in osmoregulation. In fact, the large volume of water that enters the fish body through diffusion (gill and permeable surface of pharynx) is excreted out through the kidneys. As a result the kidney faces the problem of maintaining electrolytic balance, because blood plasma has the same concentration of electrolytes as the blood ; as such urine formation begins with ultrafiltration of blood plasma. Had the filtration not been done, a lot af salts would have been lost and the fish would have toiled hard to replace those electrolytes. In fact, salts are mostly reabsorbed in kidney tubles and the urine of a freshwater fish contains much less amount of salt than its external environment or ambient water (Wedemeyer, Meyer, and Smith, 1976). Hickman and Trump (1969) have mentioned that certain freshwater fishes have no glomerulue.

The complex and specific mechanisms of ionic exchange and osmoregulation of fishes have been well dealt by Maetz (1974). However, the significant amount of ions that are lost through the huge volume of urine of freshwater fishes are replaced by diffusion of Na^+ and Cl^- through the gills as well as by absorption of food through the gut to maintain the equilbrium.

It has already been made clear that every fish prefers an optional environmental condition for its maintenance of homeostatis, growth aud reproduction. Combination of several parameters *viz*,, temperature, light, dissolved gases, physico-chemical properties, are reflected in the environmental condition and an alteration beyond acceptable limit of anv of these parameters will cause stress to the fish. Productivity of the ecosystem, which supply food to the fish, is also to be considered for the growth and maturation of the fish. In an extensive culture system productivity is of great importance (Macan, 1969 ; Odum, 1971). However, the present chapter will be restricted to other parameters of fish environment. They are discussed below :

1. *Temperature* : Fish prefers an optimal temperatue for its growth and maturation. Immune response of a fish is also dependent on temperature. Warm water mirror carps do not produce antibodies when ambient temperature is less than 12° C but cold water trouts produce antibodies when water temperature is even lower than 5° C. Roberts (1975) and Anderson and Roberts (1975) have shown that both defence mechanism and susceptibility to disease of a fish are dependent on temperature. With slightly higher than optimal temerature the wound healing of a fish is quicker. But higher temperature beyond limits would cause exhaustion for the fish. Solubility of oxygen in water is also dependent on temerature. Higher the temperature lesser is the solubility of dissolved gases. But reverse is true for the pollutants (heavy metal, pesticides and crude oils).

2. *Light* : The growth and maturation rates of fishes are also controlled by light or photoperiod. In other words, light has a primary role in food production in extensive fish culture. Excess of light stops photosynthetic action and may cause sun-burns of the fishes (Roberts, 1978).

3. *Dissolved gases* : Two dissolved gases *viz*., oxygen and nitrogen play vital role in fish life. Though concentration of oxygen in air is 260 ppm the same is quite scarce in water (0–14 ppm). Further solubility of gases in water is inversely proportional with both temperature and salinity. Though oxygen is very much needed for respiration but nitrogen is biologically inert. However, supersaturation of both these gases in water may cause

gas embolism (Rucker, 1972) for the fishes. On the contrary depletion of oxygen in water will result in asphyxia of the fishes. As oxygen is less soluble in blood plasma hemoglobin binds the same for active transport. Unloading of oxygen is accelerated by CO_2 released from tissue respiration. However, this loading and unloading of oxygen and the equilibrium maintained between O_2 and CO_2 are largely dependent on the pH of ambient water. In acid water carrying capacity of hemoglobin is reduced. Oxygen is more needed for fish during its *specific dynamic action i e.*, food intake and digestion. As such, the minimum amount of dissolved oxygen required for the good growth of fish is 5 mg/l.

Though carbon dioxide is most soluble in water yet its minute presence in water is due to less availability in air (0.04%). For healthy growth of fish 3 mg/l or less of free CO_2 is permissible in pond or hatchery waters. This high amount of free CO_2 present in water is due to chemical or biological activity. The effects of dissolved and free carbon dioxide have been reviewed by Doudoroff and Katz (1950). However, the only record of gas embolism due to CO_2 has been made by Mrsic (1933).

4. *pH, ammonia, and bicarbonates* : Best growth of fish is expected in water having a pH range between 6.7 and 8.6. But the trace elements mostly remain in available form in neutral water. It is known that NH_4^+ is harmless to fish but NH_3 is toxic. In water NH_3 combines with H_2O to form NH_4^+OH which readily dissociates to NH_4^+ and OH^- But these combination and dissociation are dependent on temperature and pH. In high pH toxic NH_3 is formed. Accroding to Trussel (1972) the total ammonia (free NH_3) in water should not exceed to 0.02 ppm for healthy growth of fish.

Similarly bicarbonate content of water is also dependent upon pH and temperature. In high pH HCO_3 quickly dissociates to $H^+ + CO_3^-$ In low pH H^+ combines with CO_3^- to from H_2CO_3. In medium to low pH H_2CO_3 can dissociate to form CO_2 and H_2O. However, according to Hart *et al.* (1945) for good growth of fish the ambient water should not have more than 180 mg/l of bicarbonates. The permissible limit of pH is 6.0—9.0, but in acid waters CO_2 attains toxic limit to cause fish mortality.

5. *Acidity* : Neess (1949) encountered hypersensitivity to bacteria of carps in acid waters (pH below 5.5). But, according to EIFAC (1968) only few experiments have been conducted to prove that fishes cultured in low pH of ambient waters are more susceptible to diseases. However, other effects of acidic waters have already been discussed.

The effects on environmental stress on the outbreaks of infectious diseases of fishes have already been discussed by Snieszko (1974) and Stankiewiez (1979). Nutritional diseases of fishes are generally encountered in intensive fish culture where wrong formulations or deficiency of vitamins are the other factors for disease manifestations (Snieszko, 1972 ; Roberts, 1978). As such the following table indicate the stress mediated disease of fishes encountered in hatcheries, raceways, a nd impoundments :

Sl. No.	Diseases	Factors associated with the diseases
1.	Furunculosis	Crowding, handling and low level of D. O. in water
2	Bacterial gill disease	Crowding, low level of D. O. and higher concentration of ammonia in water
3	Corynbacterial kidney disease	Diets containing corn-gluten and low total hardness (less than 100 ppm as $CaCO_3$) of water
4	Columnaris	Crowding, handling and higher water temperature
5	*Aromonas & Psemudomonas* hemorrhagic septicemia	Low D. O. in water, handling, crowding, prior sufferings from infection of *Costiaz* or *Trichodina ;* non-sanitary condition of ambient water together with more bacterial load ; Chronic exposure to pesticides
6	Coldwater	Low temperature of ambient water
7	Spring viremia of carps	Bad handling associated with low temperature
8	Nephrocalcinosia	Overstocking
9	Gill necrosis	Over drugging with formalin
10	Cataract of *Catla catla*	Poor sanitary condition of ambient water
11	Dropsy of carps and catfihes	Poor sanitary condition of ambient water, over stoocking and chronic exposure to low level of D. O.
12	Tumours of *Anabas testudineus*	Same as above
13	Reddish blotches of *Hypophthalmichthys molitrix*	Same as above
14	Infectious hematopoetic mecrosis	Low temperature of ambient water
15	Vibriosis	Bad handling, low level of D. O. in ambient water

Sl. No.	Diseases	Factors associated with the diseases
16	Tail and fin rot	Crowding , improper diet, temperature and chronic exposure to PCBS
17	Blue-sac	Crowding ; accumulation of nitrogenous metabolites in ambient water
18	Gas-embolism	Supersaturation of O_2, N_2 and CO_2 in water
19	Trichodinosis	Crowding, low level of D. O. in water
20	Coagulated yolk	Rough handling, mineral loss in ambient water
21	Handling loss	Repeated netting-cum-rough handling
22	Fatty infiltration of liver	Deficiency of essential fatty acids in diet
23	Improper growth	Deficiency in essential amino acids in diet, poor sanitary condition , etc.
24	Expothalmia	Deficiency of vitamin E in the diet
25	Anaemia and cloudy lens	Deficiency of riboflavin in the diet
26	Scoliosis and lordosis	Deficiency of ascorbic acid in the diet

The above table is compiled from Gopalakrishnan (1961 a and b) ; Snieszko (1972, 1973 & 1974) ; Wedemeyer, Meyer and Smith (1976) ; Chun (1976) ; Pal (1976) ; Pal & Tripathi (1978) and Schlotfeldt (1980).

Other parameters

Apart from the above mentioned diseases, mortalities of fishes due to algal toxicosis have also been reported by Matida *et al.* (1967) ; Sarig (1971) ; etc. Sewage has also been reported as a stress factor by several workers Collins, 1970 ; Heuschmann-Bruner, 1970 and Shotts *et al* 1972).

Burrows (1964), Burrows and Combs (1968), Larmoyenex and Piper (1973), Meyer and Bullock (1973), etc. have reported about the stress caused by the fish metabolites.

The effects of industrial pollution in outbreaks of infectious diseases of fishes have been reported by Snieszko *et al.* (1964) ; Simidu and Egushi (1972) ; Kusuda and Miura (1972) ; Kusuda and Yamaska (1972) ; Burton *et al.* (1972) ; Starr and Jones

(1957); Skidmore (1970); Pippy and Hare (1969); Perkins *et al.* (1972); Van Valin *et al.* (1968). Similarly effects of pesticides on the outbreaks of infectious diseases of fishes have also been reported by Couch (1974), Mohoney *et al.* (1973) and Collins (1970).

Before closing this chapter it is made clear that "Disease is the end result of an interaction between a noxious stimulus and a biological system and to understand disease is to understad all aspects of the biology of the species"—Mawdesley Thomas (1972).

Literature cited

Anderson, C. D., 1975 and Roberts, R. J., A comparison of the effects of temperature on wound healing in a tropical and a temperate teleost. *J. Fish. Bilo.*, **7** : 173-82

Burrows, R. E., 1964 Effects of accumulated excretory products on hatchery reared salmonids. *Res. Rep. U.S. Fish Wildl. Srv.*, **66** : 12-23

Burrows, R. E., 1968 and Combs, B. D., Controlled environments for Salmon propagation. *Prog. Fish. Cult.*, **30** : 123-36

Burton, D. T., 1973 Jones, A. H., and Cairns, J. Jr., Acute zinc toxicity to rainbow trout *(Salmo gairdnerii)* : confirmation of the hypothesis that death is related to tissue hypoxia. *J. Fish. Re. Bd. Can.*, **29** : 1463-66

Chun, S. K., 1976 The stress of drug treatment to common carp, *Cyprinus carpio.* (Natl. Fish. Univ. Pusan, Korea), *Abstract in Fish Health News.*, **9**(2) : 4

Collins, V. G., 1970 Recent studies of bacterial pathogens of freshwater fish. *Wat. Treat. Exam.*, **19** : 3-31

Couch, J. A., 1974 Histopathological effects of pesticides and related chemicals on the livers of fishes. *In* Symposium on Fish Pathology (Eds. Ribelin, W. E. & Nigaki, G.) Madison : University of Wisconsin Press

Doudoroff, P., 1950 and Katz, M., Critical review of literature on the toxicity of industrial wastes and their components to fish. I. Alkalies, acids and inorganic gases. *Sewage* and *Ind. Wastes*, **12** : 1432-58

EIFAC (European Inland Fisheries Advisory Commission), Report on
1968 extreme pH values and inland fisheries. *FAO U.N. Tech. Rep, No. 4*, Rome, Italy : 53 pp

Gopalakrishnan, V., Observations on a new epidemical eye diseases affecting the Indian
1961a carp, *Catla catla* (Ham. & Buch). *Indian J. Fish.*, 8(1) : 222-33

, Observations on infectious dropsy of of Indian carps and its
1961b experimental induction. *J. Sci. & Industr. Res.*, **20**(12) : 357-58

Hart, W. B., Doudoroff, P. and Greenbank, J., Evaluation of toxicity of indus-
1945 trial wastes, chemicals and other substances to freshwater fishes *Water Control Lab.* Atlantic Refining Co., Philadelphia, Penn

Heuschmann-Brunner, G., Die Aeromonaden in der Hydrobiologic. *Z. Wassor Abwasser*
1970 *Forschung*. (WAF), **3** : 40-41

Hickman, C. P. and Trump, B. F., The kidney. *In* Fish Physiology ; Ed. W. S.
1969 Hoar and D. P. Randall ; **1** : 91-239 : Academic Press, New York & London

Kusuda, R. and Miura, W., Characteristics of a *Pasteurella* sp. pathogenic for
1972 pond cultured Ayu. *Fish Pathol.*, 7(1) : 51-57

Kusuda, R., and Yamaoka, M., Etiological studies on bacterial pseudotuber-
1972 culosis in cultured yellowtail with *Pasteurella piscida* as the causative agent. I. On the morphological and biochemical properties. *Bull. Ja. Soc. Scient. Fish.*, **38** : 1325-32

Larmoyenex, J. D. & Piper, R. G., Effect of water reuse on rainbow trout in hat-
1973 cheries. *Prog. Fish. Cult.*, **35** : 2-8

Mawdesley-Thomas, L. E., Foreword. *In* Diseases of Fish. Ed. L. E. Mawdesley-
1972 Thomas. Academic Press and the Zoological Society of London. Symposia of the Zoological Society of London, No. 30 : xi-xiii

Maetz, Aspects of adaptation to hypo-osmotic and hyperosmotic
1974 environments. *In* Biochemical and biophysical perspective in marine biology. Eds. D. C. Malins and J. R. Sargent. Academic Press, **1** : 1-167

Mahoney, J. B., Midlige, F. H., and Deuel, D. G., A fin rot disease of marine and
1973 euryhaline fishes in the New York Bight. *Trans. Am. Fish. Soc.* ; **102** : 596-603

Matida, Y., 1967 — Kihura, S., Yoshimuta, C., Kumada, H., and Tokunaga, E., A toxic freshwater algae, *Glenodinium gymnodinium* Penand caused fish kills in artificially impounded Lake Sagami. *Bull. Freshwater Fish. Res. Lab.* Tokyo, Sl. No. 33 ; **17** : 73-77

Meyer, F. P., 1973 — and Bullock, G. L.. A new pathogen of channel catfish (*Ictalurus punctatus*). *Appl. Microbiol.*, **25** : 155-156

Mrisc, W., 1933 — Die Gabalasenkrankheit dev Fische. Uraschen Begleitenscheinugen und Abhlife. *Zeit. Fisch.*, **31** : 29-67

Neess, J. C., 1949 — Development and status of pond fertilization in Central Europe. *Trans. Am. Fish. Soc.*, **76** : 335-58

Odum, E. P., 1971 — Fundamentals of Ecology (3rd Ed.—Philadelphia) : Saunders

Pal, R. N., 1976 — Treatment of tumours of *Anabas testudineas* (Bloch), *J. Inland Fish. Soc.* India., **8** : 105-6

Pal, R. N., 1978 — and Tripathi, S. D., Use of terramycin for controlling fish diseases of carp and catfish culture in Indian waters. *Ibid.*, **10** : 166-68

Perkins, E. J., 1972 — Gilchrist, J. R. S. and Abbott, O. J., Incidence of epidermal lesions in fish of the North East Irish Sea. *Nature*, London ; **238** : 101-3

Pippy, J. H. C. 1969 — and Hare, G. M., Relationship of river pollution to bacterial infection in salmon *(Salmo salar)* and Suckers *(Catostomus commersonii)*. *Trans Am. Fish. Soc.*, **98** : 685-90

Roberts, R. J., 1975 — The effect of temperature on diseases and their histopathological manifestation in fish. *In* The Pathology of Fishes. Eds. W. E. Ribelin & G. Migaki, University of Wisconsis Press, Malison : 477-96

, 1978 — The pathophysiology and systemic pathology of fishes. *In* Fish Pathology. Ed. R. J. Roberts, Baillere Tindall, London : 55-91

Rucker, R. R., 1972 — Gas bubble disease of salmonids : a critical review. *W. S. Burr. Sport. Fish Wildl. Tech. Pap.*, **58** : 1-11

Sarig, S., 1971 — The prevention and treatment of warmwater fishes under sub-tropical conditions ; with special reference on intensive fish farming. *In* Diseases of fishes. Eds. S. F. Snieszko and H. R. Axelrod, T. F. H. Publishers, Neptune N. J., : 1-127

Scholotfedt, H. J., 1980 — Some clinical findings of a several years survey of intensive culture systems in Northern Germany. *In* Symposium on New Developments in the Utilization of Heated Effluents and Recirculation Systems for Intensive Culture. EIFAC, 11th Session, Norway (28-30 May) : 1-19

Simidu, U., and 1972 — Egusa, T., A re-examination of the fish pathogenic bacterium that had been reported as a *Pasteurella* species. *Bull. Jap. Soc. Scient. Fish.*, **38** : 803-12

Shotts, E. B., 1972 — Gaines, J., Martin, L., and Prestwood, A. K., *Aeromonas* induced death among fish and reptiles in eutrophic inland lake. *J. Am. Vet. med- Ass*, **161** : 603-7

Skidmore, J. F., 1970 — Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulphate. *J. exp. Biol.*, **52** : 481-94

Snieszko, S. F., 1972 — Nutritional fish diseases. *In* Fish Nutrition. Ed. J. F. Halver, Academic Press, New York and London : 403-7

, —1973 — Recent advances in scientific knowledge and developments pertaining to diseases of fishes. *Adv. Vet. Sci. Comp. Med.*, **17** : 291-314

, —1974 — The effects of environmental stress on outbreaks of infectious diseases of fishes. *J. Fish. Biol.*, **6** : 197-208

, —1964 — Bullock, G. L., Dunbar, C. E. and Petijohn, L. L., Nocardial infection in hatchery reared fingerling of rainbow trout (*Salmo gairdnerii*). *J. Bact.*, **88** : 1809-10

Stankiewies, E. B., 1979 — Fish culture in water heated by thermal effluents. *Gospodarka rybna* ; **31**(9) : 11-13 (Paper in Polish, Abstract in *Fish Health News* in English)

Starr, T. J., & 1957 — Jones, M. F., The effect of copper on the growth of bacteria isolated from marine environments. *Limnol. Oceangr*, **2** : 33-36

Steon, J. B., and 1974 — Kruysee, A., The respiratory function of the teleostean gill. *Comp. Biochem. Physiol.*, **12** : 127-42

Trussel, R. P., 1972 — The present unionised ammonia in aqueous ammonia solutions at different pH levels and temperatures. *J. Fish. Res. Bd. Can.*, **29** : 1805-7

Van Valin, C. C., 1968 — Andrews, A. K., and Eller, L. L., Some effects of Mirex on two warmwater fishes. *Trans. Am. Fish. Soc.*, 97 : 185-96

Wedemeyer, G. A., 1976 — Meyer, F. P. and Smith, L., Environmental Stress and Fish Diseases. Eds. S. F. Snieszko and H. R. Azelrod, T. F. H. Publishers, Neptune, N. J. : 1-192

CHAPTER III

Infectious bacterial diseases of fishes

Bacterial diseases of fishes are often recorded from natural resources as well as man-made impoundments particularly from waters polluted with organic load. Bacterial fish pathogens recorded from the teleosts are tabulated below :

TABLE : BACTERIAL PATHOGENS OF FISHES

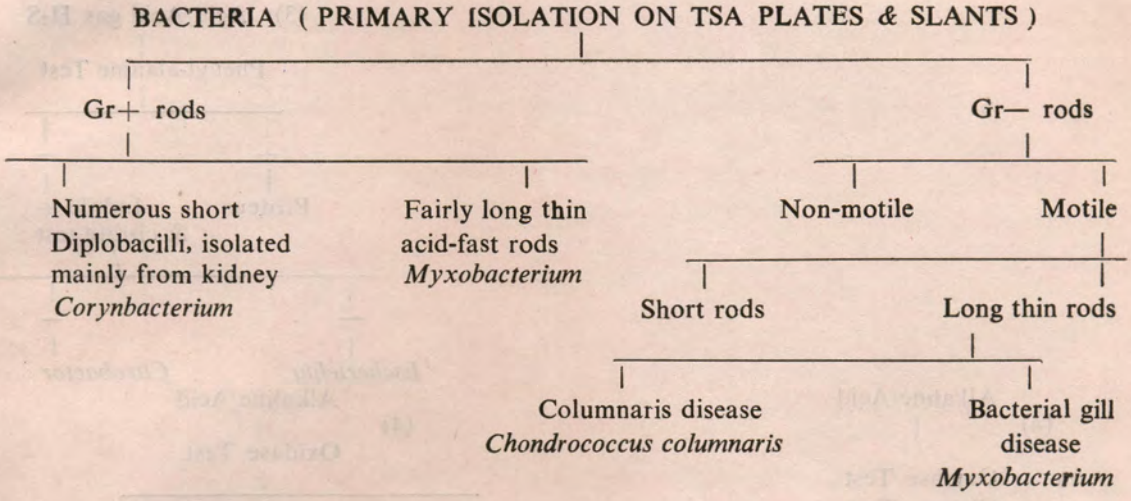
Family	Genus	Species	Habitat
Cytophagaceae	*Flexibacter*	*F. columnaris* (Bergey, 1974)	Mucus of fish
		F. psychrophila (Davis, 1946)	Fish body
Pseudomonadaceae	*Pseudomonas*	*P. fluorescens* (Plehn, 1924) Non-oxidizing Pseudomonads (Park, 1962)	Ambient soil & water ; decaying fish
Enterobacteriaceae	*Edwardsiella*	*E. tarda* (Meyer & Bullock, 1973)	Polluted waters ; Human excreta
	Unclassified	Unclassified (Bullock *et al.*, 1971) Red mouth disease (Ross *et al.*, 1966)	Unknown
Vibrionaceae	*Vibrio*	*V. anguillarum* (Cannestrini, 1893)	Marine benthos & invertebrates
	Aeromonas	*A. salmonicida* (Emerich & Weibel, 1894)	

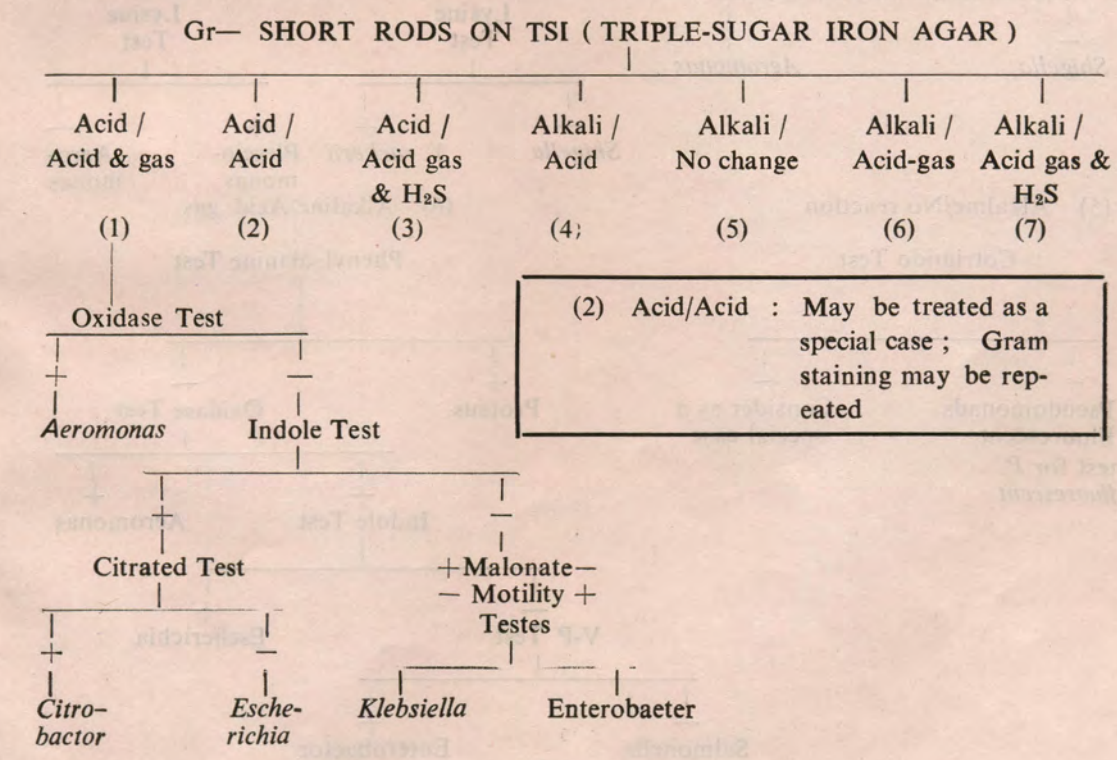
TABLE : BACTERIAL PATHOGENS OF FISHES

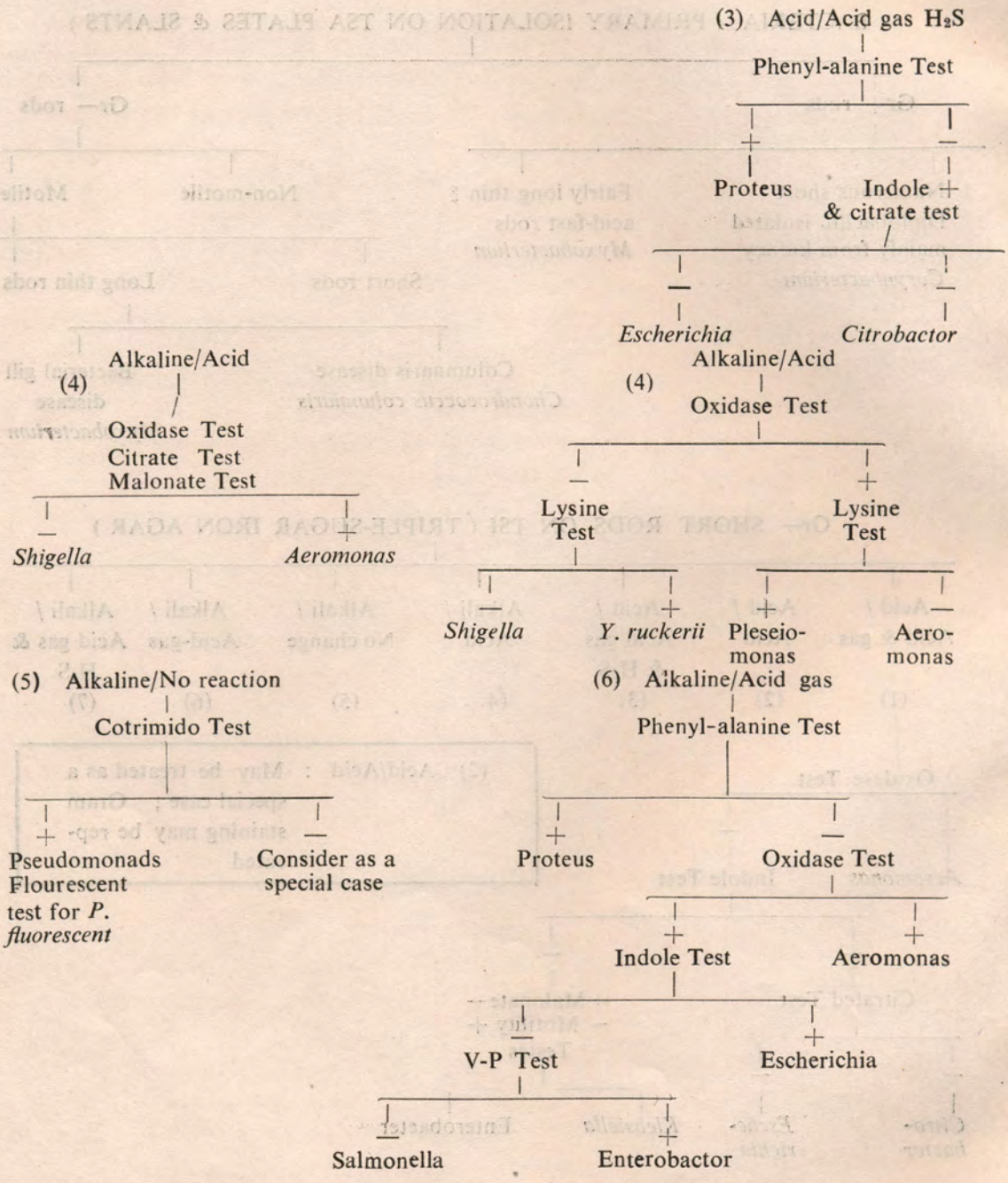
Family	Genus	Species	Habitat
		A. S. salmonicida	Freshwater fiish
		A. S. achromogens	Freshwater fiish
		A. S. nova	Freshwater fiish
		A. hydrophila (Popoff & Veron, 1976)	Freshwater fish with organic load
Uncertain	*Flavobacterium*	Flavobacter (Brisou, *et al.*, 1959 ; Kluge, 1965 ; Meyer, *et al.*, 1959 ; Snieszko, *et al*, 1964)	Amblent soil & water
	Pasteurella	*P. piscidia*	Marine fish
	Haemophilus	*H. piscicum* (Snieszko and Friddle, 1950)	Unknown
Streptococcaceae	*Streptococcus*	*Str. faecalis* (Hashina, *et al.*, 1958)	Faecal matter of warm-blooded animals
		Str. Lancefield Gr. B (Plumb *et al.*, 1974)	Estuarine fish
Bacillaceae	*Clostridium*	*Cl. betulinum*	Soil, faeces, decaying organic matter
Ccrynbacteriaceae	*Corynbacterium*	Gr+, non-sporing rods (Bolding & Merrill, 1935)	Unkonwn
Mycobacteriaceae	*Mycobacterium*	*Myco. marinum* (Bataillon & Terre, 1897)	Unknown
		Myco. fortuitum, (Ross & Broncate, 1959 ; Gordon & Smith, 1955)	Unknown

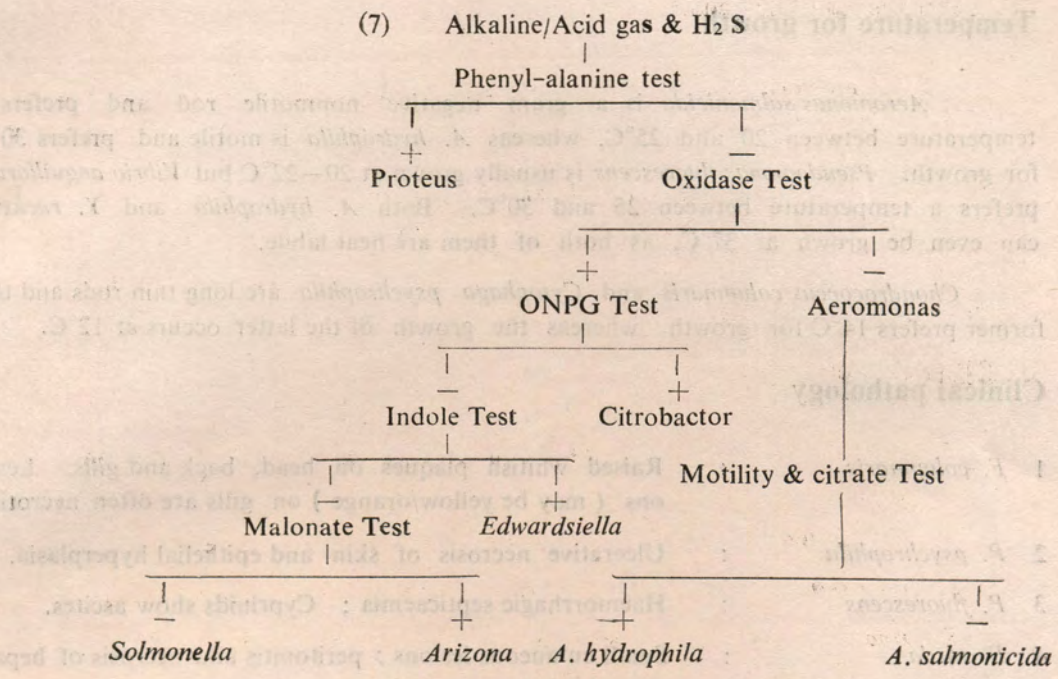
Nocardiaceae	*Nocardia*	*N. asteroides* (Valdez & Conrey, 1963)	Unknown
		N. kampachii (Kariya *et al.*, 1968)	Unknown
Chlamydiaceae		Epitheliocystis organisms (Hoffman *et al.*, 1969)	Unknown

For the presumptive identfication of bacteria pathogenic to fishes, Bullock (1971) has provided a schematis outline. However, the following scheme prepared from Bullock (1961) and Shotts and Bullock (1975) and Shotts (1976) will be useful.


BACTERIA (PRIMARY ISOLATION ON TSA PLATES & SLANTS)
Gr+ rods
Gr— rods
Numerous short Diplobacilli, isolated mainly from kidney
Corynbacterium
Fairly long thin acid-fast rods
Myxobacterium
Non-motile
Motile
Short rods
Long thin rods
Columnaris disease
Chondrococcus columnaris
Bacterial gill disease
Myxobacterium


Gr— SHORT RODS ON TSI (TRIPLE-SUGAR IRON AGAR)
Acid / Acid & gas
(1)
Acid / Acid
(2)
Acid / Acid gas & H2S
(3)
Alkali / Acid
(4)
Alkali / No change
(5)
Alkali / Acid-gas
(6)
Alkali / Acid gas & H2S
(7)
Oxidase Test
+
—
Aeromonas
Indole Test
+
—
Citrated Test
+Malonate—
— Motility +
Testes
+
—
Citro-bactor
Esche-richia
Klebsiella
Enterobaeter
(2) Acid/Acid : May be treated as a special case ; Gram staining may be repeated


(3) Acid/Acid gas H_2S
Phenyl-alanine Test
+
−
Proteus
Indole + & citrate test
−
−
Escherichia
Citrobactor
(4) Alkaline/Acid
Oxidase Test
Citrate Test
Malonate Test
−
+
Shigella
Aeromonas
(4) Alkaline/Acid
Oxidase Test
−
+
Lysine Test
Lysine Test
−
+
+
−
Shigella
Y. ruckerii
Pleseiomonas
Aeromonas
(5) Alkaline/No reaction
Cotrimido Test
+
−
Pseudomonads Flourescent test for *P. fluorescent*
Consider as a special case
(6) Alkaline/Acid gas
Phenyl-alanine Test
+
−
Proteus
Oxidase Test
+
+
Indole Test
Aeromonas
−
+
V-P Test
Escherichia
−
+
Salmonella
Enterobactor


(7) Alkaline/Acid gas & H_2S
Phenyl-alanine test
+
Proteus
—
Oxidase Test
+
ONPG Test
—
Aeromonas
—
Indole Test
+
Citrobactor
—
Malonate Test
+
Edwardsiella
Motility & citrate Test
—
Solmonella
+
Arizona
+
A. hydrophila
—
A. salmonicida

Temperature for growth

Aeromonas salmonicida is a gram negative nonmotile rod and prefers a temperature between 20 and 25°C, whereas *A. hydrophila* is motile and prefers 30°C for growth. *Pseudomonas fluorescens* is usually grown at 20—22°C but *Vibrio anguillarum* prefers a temperature between 25 and 30°C. Both *A. hydrophila* and *Y. ruckerii* can even be grown at 37°C, as both of them are heat labile.

Chondrococcus columnaris and *Cytophaga psychrophila* are long thin rods and the former prefers 14°C for growth, whereas the growth of the latter occurs at 12°C.

Clinical pathology

1	*F. columnaris*	:	Raised whitish plaques on head, back and gills. Lesions (may be yellow/orange) on gills are often necrotic.
2	*P. psychrophila*	:	Ulcerative necrosis of skin and epithelial hyperplasia.
3	*P. fluorescens*	:	Haemorrhagic septicaemia ; Cyprinids show ascites.
4	*E. tarda*	:	Small cutaneous lesions ; peritonitis and necrosis of hepatic and renal tissue.
5	E. R. M. bacterium	:	Erosion of the lower jaw, necorsis of intestinal mucosa, deep seated haemerrhage of the tissues of head.
6	*V. anguillarum*	:	Anorexia, darkening and sudden death. Generalised septicaemia.
7	*A. hydrophila*	:	Red irregular haemorrhages on body surface, base of fins and ascites. Haemorrhages over viscera.
8	*A. salmonicida*	:	General septicaemia, darkening, anorexia. Haemorrhages at the base of fins and on the gills.
9	Flavobacterium	:	Haemorrhagic septicaemia or chronic granulomatous disease.
10	*H. piscicum*	:	Small white epidermal spongiotic hyperplasia ; followed by an ulcer with a white rim.
11	*Pasteurella*	:	Acute haemorrhagic septicaemia ; lesions on haematopoetic tissue.

12	*Streptococcus faecalis*	:	General septicaemia.
13	*Clostridium betulinum*	:	Nervous imbalance.
14	KD corynbacterium	:	Darkening ; Exophthalmos and small haemorrhages.
15	*M. marinum*	:	Darkening and swelling of abdomen.
16	*M. fortuitum*	:	Darkening and hypersensitivity.
17	*Nocardia*	:	Anorexia, emaciation, and distension of the mouth.

Control measures

Control measures generally adopted against bacterial diseases of fishes are sanitation and chemotherapy (Shotts & Snieszko, 1976). Sulfonamides (Snieszko *et al.*, 1950), Oxytetracyclines (Snieszko *et al.*, 1952) and Nitrofurans (Post, 1959) are the drugs commonly used for disease treatments. Immunization and genetic selection are also practised against infection of *Vibrio anguillarum* (Rohovec, *et. al.*, 1975, Gjodram and Aulestad, 1974). No treatment method is available against the infections of *Flavobacterium*, *Streptococcus* Lancefield group B, *Mycobacterium* spp. and *Nocardia*.

Before closing this chapter it is suggested that one should bear in mind that fishes are poikilothermal animals, and the bacteria isolated from fishes as such should be grown according to their ambient temperature. Often a common mistake is made by incubating the bacteria at 37°C which is the average human body temperature. At this temperature most of the fish pathogenic or symbiotic bacteria cannot be isolated because the differenc of temperature between ± 5°C (compared to ambient) may be lethal for the bacteria desirable for isoclation. Further, even recent edition of "Bargey's Manual" is not a full proof book for identifying 'Fish Bacteria', as the same incorporates mostly the bacteria isolated from warm blooded animals (perhaps the only exception is *Aeromonas salmonicida*). Recent advancement in sero-dignosis of fish diseases is presented in the last chapter for use.

Literature cited

Bataillon, E. and Terre, L., 1897 Un nouveau type detuberculese. *C. r. Seans. Soc.* Biol., **49** : 446-449 (not seen in original)

Belding, D. L. and Merrill, B., A preliminary report upon a hatchery disease of the 1935 Salmonidae. *Trans. Am. Fish. Soc.*, **65** : 76-84

Bergey, D. H., *Bergey's Manual of Determinative Bactereology*. Ed. R. E. Buchanon and N. E. Gibbens, VIII ; Williams and Wilkins, Boltimore : 1974 1094 pp

Briseu, J., Tysset, C. and Vacher, B., Reserches sunles Pseudomonadaceae. 1959 Etudes de souches de Flavobacterium isoleas des poissons de l'eau douce. *Annls. Inst. Pasteur, Paris.*, **96** : 633 (not seen in original)

Bullock, G. L., A schematic outline for the presumptive indentification of bacterial 1961 diseases of fish. *Prog. Fish. Cult.* (October), **23** : 147-151

— , Identification of fish pathogenic bacteria. *In Diseases of Fishes.* 1971 Ed. S. F. Snieszko and H. P. Axelrod, Book 2B ; Neptune, N. J., TFH Publishers : 1-41

Bullock, G L., Conroy, D. A. and Snieszko, S. F., Bacterial. *Diseases of Fishes.* 1971 Ed. S. F. Snieszko and H. P. Axelrod, Book 2A ; Neptune, N. J., TFH Publishers : 1-151

Canestrini, G., La malattia dominanto della anguillo. *Atti. Ist. Venets Sci.*, 1893 **51** : 809-814 (not seen in original)

Davis. H. S., Care and diseases of trout. *Res. Rep. U.S. Fish Wildl. Serv*, No. 12 1946

Emmerich, R. and Weibel, E., Uber eine dursh Bacterian erzeugte souche unter den 1894 Ferellen. *Arch. Hyg. Bakt.*, **21** : 1-24 (not seen in original)

Gjodram, T. and Aulestad, D., Selection experiments with Salmon. I. Differences 1974 in resistence to vibrio disease in Salmon parr (*Salmo salar*). *Aquaculture* ; **3** : 51-59

Gordon, R. E. and Smith, E. E., Rapidly growing acid fast bacteria. II. Species 1955 description of *Mycobacterium fortuitum*. *J. Bact.*, **69** : 502-557

Hoffman, G. L., Dunbar, C. E., Wolf, K. and Zwillondbrg, L. O., Epitheliocystis 1969 a new disease of the bluegill *(Lepamis macrochiurus), Anatania Von Leeuwenhoek ;* **35** : 146-158

Hoshina. T., Sano, T. and Morimoto, Y., *Streptococcus* pathogenic to fish. 1958 *J. Tokyo Univ. Fish.*, **44** : 57-68

Kariya, T., Kubota, S., Nakamura, Y. and Kira, K., Neocardial infection in cultured
1968 yellowtails (*Seriola quinquevadiata* and *S. purpurescens*). I. Bacteriological study. *Fish Path.*, **3** : 16-23

Kluge, J. P., A granulemateus disease of fish produced by *Flavobacteria*. *Pathologia*
1965 *Vet.*, **2** : 545-552

Meyer, F. P. and Bullock, G. L., *Edwardsiella tarda*, a new pathogen of channel catfish
1973 (*Ictalurus punctatus*). *Appl. Microbiol.*, **25** : 155-156

Meyers, S. P., Baslow, M. H,, Bein, S. J, and Marks, C, E., Studies of *Flavobacterium*
1959 *piscida*. I. Growth, Toxicity and ecological considerations. *J. Bact.* 78 : 225-230

Park. R. W. A., A study of certain heterotrophic polary flagellated Naten bacteria :
1962 *Aeromonas*, *Pseudomonas*, and *Laphomonas*. *J. Gen. Microbiol.*, **27** (1) : 121-133

Plehn, M., Praktikum den Fischkrankheiten. Stugart : Schweizorbart (not in seen
1924 original)

Plumb, J. A., Schachte, J. H., Gaines, J. L., Pettier, W. and Carroll, B., *Streptococ-*
1974 *ccus* spp. from marine fishes along the Alabama and North-West Florida coast of the Gulf of Mexico. *Trans. Am. Fish. Soc.*, 103 : 358-361

Popoff, M. and Veron, M., A taxonomic study of the *Aeromonas hydrophila—A. punctata*
1976 group. *J. Gen. Microbiol.*, **94** : 11-22

Post, G., A preliminary report on the use of nitrofuran compounds for furunculosis of
1959 trout with special emphasis on furoxone. *Prog. Fish. Cult.*, **21** : 30-33

Rohovec, J. S., Garrison, R. L. and J. L. Fryer, Immunization of fish for the control of
1975 vibriosis. *Proc*, Third U.S.-Japan Aquaculture meeting. *Spl. Publ. Jap. Sea. Reg. Fish. Res. Lab.*, 105-112

Ross, A., and Broncate, F., *Mycobacterium fortuitum* from the tropical fish, *Hyphoseabryon*
1959 *inneei*. *J. Bact.*, **78** : 393-395

Ross, A., Rucker, R. R. and Ewing, W. H., Description of bacterium associated with a
1966 redmouth disease of *Salmo gairdnerii*. *Can. J. Microbiol.*, **12** : 763-770

Shotts, E. B., Unknown culture of gram negative rod : Identification of fish pathogenic
1976 bacteria (*personal communication*)

Shotts, E. B. and Bullock. G. L., Bacterial diseases of fish : diagnostic procedures for
1975 gram negative pathogens. *J. Fish. Res. Bd. Can.*, **32** : 1243-1247

Shotts, E. B. and Snieszko, S. F., Selected bacterial fish diseases. *In* Wildlife Diseases. 1976 Ed. L. A. Page ; Planum Publishing Corpn., New York : 143-150

Snieszko, S. F. and Friddle, S. B., A contribution to the aetiology of ulcer diseases of 1950 trout. *Trans. Am. Fish. Soc.*, **78** : 56-63

Snieszko, S. F., Gutsell, J. S. and Friddle, S. B., Various sulfonamide treatments of 1950 furunculosis on *Salvenilus fontanalis*. *Trans. Am. Fish. Soc.*, **78** : 181-188

Snieszko, S. F., Griffin, P. J. and Friddle, S. B., Antibiotic treatment of ulcer diseases 1952 and furunculosis in trout. *Trans. N. Amer. Wildl. Conf.*, **17** : 197–213

Snieszko, S. F., Bullock, G. L., Hollis, E. and Boone, J. G., *Pasteurella* Sp. from an 1964 epizootic of white perch (*Roccus americanus*) in Chespeake Bay tidewater areas. *J. Bact.*, **88** : 1814-1815

Valdez, I. and Conroy, D. A., The study of a tuberculosis—like condition in neon tetras 1963 (*Hyphoseabryon inneei*) ; II Characterization of the bacteria isolated *Microbiologia Spl.*, **16** : 249-253

CHAPTER IV

Infectious viral diseases of fishes

A comprehensive account of fish diseases of viral origin was first made available by Jensen (1978), but a more comprehensive and systematic account of fish viruses has been recently presented by Mc Allister (1979). It is needless to mention that fish virology is a most modern subject and with the advent of fish cell-lines and tissue culture in the latter half of the present century a great advancement has been made. However, more studies are yet to be made as studies on this branch of science are mostly confined in countries like U. K., U. S. A. and Japan.

All fish viruses have not been studied thoroughly ; as such some are well characterized while others need isolation. However, fish viruses can be tentatively classified as follows :

1. *Herpesviridae*

Isolated in cell-culture	a	Channel catfish virus	Wolf and Darlington, 1971 ; Fijan, 1968
	b	*Herpesvirus salmonis*	Wolf, *et al.*, 1975a, b; Wolf & Taylor, 1975
	c	Nerka virus	Sano, 1976
Herpesvirus like agents	a	Epithelioma papillosum of *cyprinus*	Bauer *et al*., 1969
	b	*Herpesvirus scophthalmi*	Buchanan & Medley, 1978

2. *Reoviridae*

Isolated in cell-culture	a	Infectious pancreatic necrosis virus	Wolf *ei. al.*, 1960
	b	Eel virus european	
	c	Golden shiner virus	Mitchell & Plumb, 1980

3. *Iridoviridae*

Isolated in cell-culture	a	Lymphocystis virus	Dunbar & Wolf, 1966
	b	Piscine erythrocytic necrosis virus	Laird & Bulloek, 1969

4. *Rhabdoviridae*

Isolated in cell-culture	a	Infectious haematopoetic necrosis virus	Amend, *et al.* 1999
	b	Egtved virus	Jensen, 1963
	c	Rhabdovirus carpio	Fijan, *et al.*, 1971
	d	Pike-fry rhabdovirus	deKinkelin, *et al.*, 1973
	e	Eel virus-american	Mc Allister, *et al.*, 1977
	f	Rhabdovirus of European eel	Sano, 1976 ; Sano, *et al* , 1977

5. *Retroviridae*

a	Atlantic salmon fibrosarcoma	Duncan, 1978
b	Esox lymphosarcoma	Papas, *et al.*, 1976 ; Winqvist *et al.*, 1973
c	Esox epidermal hyperplasia	Winqvist *et al.*, 1968 ; Sonstegard, 1976 ;
d	Walleye dermal sarooma	Walker, 1961, 1969
e	Walleye epidermal hyperplasia	Walker, 1969, Yamamoto, *et al.*, 1976
f	White sucker epidermal papilloma	Sonstegard, 1977a, b

6. Unclassified fish viruses causing neoplasia

a	Stomatopapilloma of eel	Mc Allister, *et al.*, 1977
b	Brown bullhead papilloma	Edwards, *et al.*, 1977
c	Atlantic Salmon papilloma	Carlisle, 1976, 1977 ; Wiren, 1971
d	Pleuronoctid epidermal papilloma	Brooks, *et al.*, 1969

7 Unclassified putative fish viruses

Isolated in cell-culture	a	Bluegill virus	Hoffman, *et al.*, 1969
	b	Grunt-fin agent	Clem, *et al.*, 1965
	c	Ulcerative dermal necrosis virus	Roberts, 1972
	d	Gill necrosis of carp virus	Popkova & Shohelkunov, 1978

8 Susceptible cell system

a Rainbow trout gonad (RTG-2)	Infectious pancreatic necrosis virus Eel virus european *Herpesvirus salmonis* Eel virus american Infectious haematopoetic necrosis virus Egtved virus Stomatopapilloma of eel virus Eel viruses of Japan	
b Brown bullhead c Channel catfish ovary	Channel catfish virus	
d Chinock salmon embryo	Infectious pancreatic necrosis virus *Herpesvirus salmonis* Infectious haematopoetic necrosis virus	
e Fat-head minnow	Golden shiner virus Pike-fry rhabdovirus Eel virus japan (I & II) Rhabdovirus carpio Stomatopapilloma of eel virus	

f Bluegill fry — Lymphocystis virus

g Other cell-lines (certified) with their abbreviations

Bluegill (BGL)
Channel catfish ovary (CCO)
Coho salmon embryo (CSE)
Common carp hyperplasia (EPC)
Bluestripped grunt (GF)
Large-mouth bass (LBF)
Rainbow trout fry (RTF)
Sockeye salmon (SSE)
Steelhead trout embryo (STE-137)
Red-sword tail (SWT)
Carp sac fry (CSF) (Horiuchi *et al.*, 1979)

9 Optimum temperature for cell-culture and cytopathetic effect (site of replication)

a	Infectous haematopoetic necrosis virus	12-18°C	
	Pike-fry rhabdovirus	21-28°C	Lytic
	Egtved virus	14-15°C	
	Rhabdovirus carpio	20-22°C	
	Eel virus american	20-27°C	(cytoplasm)
	Rhabdovirus of European eel	15°C	
b	Channel catfish virus	25-30°C	Syncetium
	Herpesvirus salmonis	4-10°C	
	Herpesvirus scophthalmi		Nucleus
	Epithelioma papillosum of cyprinus		
	Nerka virus		
c	Infectious pancreatic necrosis virus	15°C Lytic	Cyto-
	Eel virus european	20°C Lytic & Pyknosis	plasm
	Golden shiner virus	30°C Syncetium	

d	Piscine erythrocytic ncrosis virus Lymphocystis virus	25°C Hypertrophy	Cytoptasm
e	Eel virus (Stomatopapilloma)	16-20°C	Lytic
f	Eel virus japan (II)	20-25°C	Syncetium (Cytoplasm)

Most of the above mentioned viral agents generally affect fry and fingerlings of fishes. Spring virus of carps (SVS) and fish pox virus (EV) infect even adults. Same is true for PENV (Piscine erythrocytic necrosis virus) as well.

10. Clinical signs

I *RNA virus diseases*

a IPNV :— Darker in colour, tail chasing, exophthalmia, distended abdomen ;

b VHS :— Darker in colour, haemorrhages at the base of fins and in gills, lethargy ;

c IHNV :— Lethargy-cum-sporadic hypersensitivity, darker in colour, exophthalmia, distended abdomen, long white faecal casts ;

d SVC :— Darker in colour, petechial haemorrhages, loss of balance, gathering near outflows, exophthalmia, dropsy ;

e PFR :— Loss of balance, swimming near the surface, haemorrhages of skin and gills, cranial distension and exophthalmia.

II *DNA virus diseases*

a CCV :— Loss of balance, hanging vertically in the water, abdominal distension and haemorrhages of skin and gills ;

b *Herpesvirus salmonis* :— Exophthalmia, darker in colour, distended abdomen and anaemia.

Other viruses generally cause papillomatosis. Carp pox lesions are raised white nodules. PENV infects both mature and immature erythrocytes resulting in anaemia of the diseased fish.

Control measures generally taken against viral diseases of fishes are avoidance of an infection and chemotherapy with synthetic polynucleotides (Roberts, 1978). Raising the temperature of ambient water controls IHNV infection (Amend, 1970). Immunization of the stocking material with sonicated antigens or avirulent strains (alive antigen) may also be used. Mass vaccination can be done by immersing the stocking material in hyperosmotic solutions containing the antigen (Amend and Fender, 1976). Recently, more efforts are being made to produce bacterins and vaccines on commercial scale to control infectious diseases of fishes (Fryer *et al.*, 1977).

Before close it must also be mentioned that guidelines for virological examination of fishes have been suggested by Wolf (1970). Wolf and Quimby (1977 & 1978) have provided much information on—(a) primary monolayer culture of fish cells initiated from minced and trypsinized tissues, (b) procedures for subculturing fish cells and propagating fish cell-lines, and (c) systematic management of animal cell-lines.

Literature cited

Amend, D. F., 1970. Control of infectious haematopoetic necrosis virus disease by elevating the water temperature. *J. Fish. Res. Bd. Can.*, **27** : 265-270

Amend, D. F. & Fender, F. C., 1976. Uptake of bovine serum albumin by rainbow trout from hyperosmotic solution : a model for vaccinating fish. *Science*, **192** (4241) : 793-794

Amend, D. F., Yasutake, W. T. and Mead, R. W., 1969. A haematopoetic virus disease of rainbow trout and sockeye salmon. *Trans. Am. Fish. Soc.*, **98** : 796-804

Bauer, O. N., Musselius, V. A. and Strelkon, Yu. A., 1969. Diseases of pond fishes (translated from Russian). *Israel Program for Scientific Translations*, Jerusalem, TT-72-50070 (1973) : 220 pp

Brooks, R. E., McArn, G. E. and Wellings, S. R., 1969. Ultrastructural observations on an unidentified cell type found in epidermal tumours of flounders. *J. Natl. Cancer Instt.*, **43** : 97-109

Buchanan, J. S. and Madeley, C. R., 1978 Studies on *Herpesvirus scophthalmi* infection of turbat, *Scophthalmus maximum* I. Ultrastructural observations. *J. Fish. Dis.*, I : 283-285

Carlisle, J. C., 1976 A study of epithelioma in the Atlantic Salmon (*S. salar*). *In* Wildlife Diseases, Ed. L. A. Page, Plenum Publishers, New York : 443-444

— , 1977 An epidermal papilloma of the Atlantic Salmon (*S. salar*)-II. Ultrastructure and aetiology. *J. Wildi. Dis.*, **13** : 235-239

Clem, I. W., Siegal M. M. and Priis, R. R., 1965 An orphan virus isolated in marine fish cell tissue culture. *Ann. N. Y. Acad. Sci.*, **126** : 343-361

Dunbar, C. E. and wolf, K., 1966 The cytological course of experimental lymphocystis in the bluegill. *J. Infect. Dis.*, **116** : 466

Duncan, I. B., 1978 Evidence for an oncovirus in swimbladder fibrosarcoma of Atlantic Salmon (*S. salar*). *J. Fish. Dis.*, **1** : 127

Edwards, M. R., Samsonaff. W. A. and Kuzia, E. J., 1977 Papiloma like viruses from catfish. *Fish Health News*, **6** : 94-95

Fijan, N. N., 1968 Progress report on acute mortality of channel catfish fingerlings caused by a virus. *Bull. Offs. int. Epizoot.*, **69** : 1167-1168

Fijan, N. N., Petrinec, I., Sultimanovie, D. and Zwillenberg, L. O., 1971 Isolation of viral causative agent form the acute form of infectious dropsy in carp. *Vet. Arh.*, **41** : 123-135

Fryer, J. L., Amend, D. F., Harroll, I. W., Novatny, A. J., Plumb, J. A., Rohovec, J. S., 1977 and Tebbit, G. L., Development of bacterins and vaccines for control of infectious diseases in fish. *In* Oregon State University Sea Grunt College Program, Publ. No ORESU-T-77-012 : 10 pp

Hoffman, G. L., D unbar, C. E., Wolf, K. and Zwillenberg, L. O., 1969 Epitheliocystis—a new disease of the bluegill (*Lepomia macrochiurus*). *Anatomia Von Leenwenhoek*, **35** : 146-158

Horiuchi, M., Nakata, M. and Kohga, K., A new cell-line from carp sac-fry. *Bull. Jap. Soc. Sci. Fish.*, **45** (2) : 147-151

Jensen, M. H., 1963 Preparation of fish tissue cultures for virus research. *Bull. Offs. int. Epizoot.*, **59** : 131-134

Jensen, W. J., 1978 Fish diseases of viral origin. A schematic review. *Nord. Veterinarmed.*, **30** (4 & 5) : 217-220

deKinkelin, P., Bootzma R., and Galimard, B., Isolation and identification of causative 1973 agent of reddisease of pike. *Nature*, London, **241** : 465-467

Laird, M. and Bullock, W. L., Marine fish haematozoa from New Brunswick and New England. *J. Fish Res. Bd. Can.*, **26**, 1075

McAllister, P. E., Fish viruses and viral infections. *In* Comprehensive Virology. Ed. 1979 H. F. Conrat and R. R. Wagner. Plenum Pubg. Corpn : 401-470

— , Nagabayashi, T. and Wolf, K., Viruses of eels with and without stoma- 1977 topapillomas. *Ann. N. Y. Acad. Sci.*, 298 : 233

Mitchell, A. J. and Plumb, J. A., Toxicity and efficacy of Furanace on channel catfish in- 1980 fected experimentally with *Aeromonas hydrophila*. *J. Fish. Dis.*, **3**(2) : 93-99

Papas, T. S., Dahlberg, J. E. and Sonstegard, R. A., Type C. virus in lymphosarcoma in 1976 northern pike *(Esox lucius)*. *Nature*, London, **261** : 506

Popkova, T. I. and Shehelkunov, I. S., Vedelenie virusa of Karpoy, bol'nykh zhabernym 1978 nekrozom. *VNIIPRKII Rybn. Khoz.*, **4** : 34

Roberts, R. J., Ulcerative dermal necrosis (UDN) of salmon *(S. salar)*. *In* Diseases of 1972 Fish. Proc. Symp. No. 30. Zoological Society, London. Academic Press and the Zoological Society : 53-81

— , The virology of teleosts. *In* Fish Pathology. Ed. R. J. Roberts. 1978 Baillere Tindall, London : 114-143

Sano, T., Viral diseases of cultured fishes in Japan. *Fish. Pathol.*, **10**(2) : 221-226

Sano, T., Nishimura, T., Okamoto, N. and Fukuda, H., Studies on viral diseases of 1977 Japanese fishes. VII. A rhabdovirus isolated from European eel, *Anguilla anguilla*. *Bull. Jap. Soc. Sci. Fish.* No. 43 : 491

Sonstegard, R. A., Studies on the etiology and epizootiology of lymphosarcoma in Esox 1976 (*Esox lucius* L. and *E. masquinongy*). *Prog. Exp. Tumor. Res.*, **20** : 141

— , The potential utility of fishes as indicator organisms for environmental 1977a carcinogens. *In* Wastewater Renovation and Reuse. Ed. F. M. D. Itri Dekkar, New York : 561-567

— , Environmental carcinogenesis studies in fishes of the Great Lakes of 1977b North America. *Ann. N.Y. Acad. Sci.*, **298** : 261

Walker, R., Fine structure of a virus tumor of fish. *Ann. Zool.*, **1** : 395-396 1961

— , Virus associated with epidermal hyperplasia in fish. *Natl. Cancer Inst. Monogr.* No. 31 : 195 1969

Winqvist, G., Ljungberg, O. and Hellstroem, B., Skin tumours of northern pike (*Esox lucius*). II. Viral particles in epidermal proliferations. *Bull. Offs. int. Epizoot.*, **69** : 1023-1031 1968

Winqvist, G., Ljungberg, O. and Ivarsoon, B., Electron microscopy of sarcoma of the northern pike (*Esox lucius* L.). *Bibl. Haematal.*, **39** : 26-28 1973

Wiren, B., Wart disease in Atlantic salmon (*Salmo salar*. L.) . Histological studies of epidermal papillomas of reared salmon. *Rep. Swedish Salmon Res. Instt.*, Sweden, No. 7 : 4 pp 1971

Wolf, K. and Darlington, R. W., Channel catfish virus. A new herpesvirus of ictalurid fish. *J. Virol.*, **8** : 525-533 1971

Wolf. K. and Quimby, M. C., Primary monolayer culture of fish cells initiated from minced tissues. *Tissue Culture Association*—Procedure No. 41125 : 445-448 1977

— , Primary monolayer culture of fish cells initiated from trypsinized tissues. *Ibid.*, Procedure No. 41541 : 453-456 1977

— , Procedures for subculturing fish cells and propagating fish cell-lines. *Ibid.*, Procedure No. 49145 : 471-474 1977

— , Systematic management of animal cell-lines. *Ibid.*, Procedure No. 40091 : 741-744 1978

Wolf, K. and Taylor, W. G., Salmonid viruses : a syncytium forming agent from rainbow trout. *Fish Health News*, **4** : 3 1975

Wolf, K., Sano, T. and Kimura, T., Herpesvirus disease of salmonids. *Fish Wildl. Serv. Fish Disease Leafl.* No. 44 : 8 pp 1975a

Wolf, K., Herman R. L., Darlington, R. W. and Taylor, W. G., Salmonid viruses : Effects of *Herpesvirus salmonis* in rainbow trout fry. *Fish Health* News, **4** (3) : 8 1975b

Yamamoto, T., Macdonald, R. D., Gillespie, D. C. and Kelly, R. K,. Viruses associated with lymphocystis disease and dermal sarcoma of walleye (*Stizostedion vireum vireum*). *J. Fish. Res. Bd. Can.*, **33** : 2408 1976

CHAPTER V

Serodiagnosis of fish diseases

Recent advent of serodiagnosis fish diseases has helped much for standardization of the diagnosis. Being more specific these biological tests are most subjective and certain Serotechniques generally used in the identification of fish diseases are :

a Precipitin test ;
b Agglutination test ;
c Immune diffusion test ;
d Passive haemagglutination test ;
e Fluorescent antibody test (FAT) ;
f Serum-viral neutrilization test.

However, all these antibody-antigen reactions are subject to pH, temperature and time of incubation.

Serodiagnoses applied in diseases of fishes

A *Infectious pancreatic necrosis virus (IPNV)*

a New Zealand Rabbit—IPNV antiserum has no cross reaction with rabbit anti VHS (Jørgensen, 1969) ;
b Complement fixation test for IPNV (Finlay & Hill, 1975) ;
c Fluorescent antibody test (Tu *et al.*, 1974) ;
d Cross reaction between Sp and Ab strains of IPNV in direct FAT (Jørgensen, 1972) ;
e Specific neutralizing antibody (Wolf & Quimby, 1969)
f Anti-IPNV antibodies are Ig M like immunoglobulins (Jørgensen, 1973) ;
g Plaque morphology in RTG_2 cells (Wolf, 1973) ;
h Five strains belonging to forty-two IPNV isolants (Livents & Springer, 1973) and
i Detection of IPNV (McCarthy, 1975).

B *Infectious haematopoetic necrosis virus (IHNV)*

a No antigenic relationship between IPNV and Egtved virus (McAllister *et al.*, 1974a) ;

b Low cross reaction among IHNV, PFR, and SVC (Hill *et al.*, 1975) ;

c FAT and indirect fluorescent antibody staining (McAllister *et al.*, 1974b)

d Identification of IHNV (Wolf *et al.*, 1973) ;

e Haematological and blood chemical changes (Amend & Smith, 1975)
and

f Auto-interference of IHNV (McAllister & Pilcher, 1974).

C *Channel catfish virus (CCV)*

a Virus neutrilizing activity (Plumb, 1973) ;

b Quantifying CCV or antibody (Gratzek *et al* , 1973) ;

c Immune response and antibody characterization (Heartwell, 1975)—Acrylymide gel electrophoresis of serum
and

d Passive cutaneous anaphylaxis (PCA) Heartwell & Panley, 1975).

D *Viral haemorrhagic septicaemia virus (VHSV)*

a Fluorescent antibody technique (FAT) to demonstrate antigens (Jørgensen and Meyling, 1972) ;

b Specific neutrilizing antibody (Jørgensen, 1972)
and

c No antigenic relationship with IHNV and IPNV (McAllister *et al.*, 1974a).

E *Bluegill virus*

a No cross reaction with viruses causing influenza, mumps, and lymphocytic chorionmeningitis (Backwith, 1974).

F *Swim-bladder infection of carp*

a Cross reaction with Rhabdovirus carpio (Backman & Ahne, 1973).

G *Aeromonas salmonicida*

a Positive-agglutination with rabbit anti- *A. salmonicida* (Rabb *et al.*, 1964) ;

b Auto-agglutination of virulent strain (Bullock, 1976) ;

c McCarthy's latex agglutination to identifying *Aeromonas salmonicida* ;

d Indirect FAT for identification (Klontz and Anderson, 1970)

and

e Precipitin and agglutinin tests (Bullock, 1966).

H *Aeromonas hydrophila*

a Serological heterogenecity of strains (Bullock, 1976).

I *Pseudomonas fluorescens*

a Fluorescent test (not a serological test).

J *Enteric red mouth* (*Yersinia ruckerii*)

a Indirect FAT for confirmatory identification (Bullock, 1976) ;

b Slide agglutination for confirmatory identification (Bullock, 1976)

and

c Oral immunization (Ross and Kontz, 1965).

K *Vibrio anguillarum*

a Two serotypes cause fish diseases (Bullock, 1976) ;

b Slide agglutination for identification (Bullock, 1976)

and

c Indirect FAT for presumptive identification and confirmatory diagnosis of isolates (Bullock, 1976).

L *Edwardsiellatarda*

a Availability of diagnostic antiserum (Bullock, 1976).

M *Corynbacterium kidney disease*

a Double diffusion test for identification (Bullock *et al.*, 1974) ;

b Indirect FAT for confirmatory identification (Bullock, 1976) ,

c FAT for identification (Bullock & Stuckey, 1975)

and

d Serological diagnosis (Chen *et al.*, 1974).

Literature cited

Amend, D. F. and Smith, 1975 Patho-physiology of infectious haematopoetic necrosis virus disease in rainbow trout : haematological and blood chemical changes in moribund fish. *Infect. Immun.*, **11** : 171-179

Backnon. P. A and Ahne, W., 1973 Isolation and characterization of agent causing swim-bladder inflammation in carp. *Nature*, London ; **244** : 235-237

Backwith, W., 1974 Characterization and intracellular replication of the bluegill virus. *In* Ph. D. Thesis ; Lehigh University, Bethlehem, Pa 159 pp

Bullock, G. L., 1966 Precipitin and agglutinin reactions of Aeromonads isolated from fish and other sources. *Bull. Offs. int. Epizoot.*, **65**(5-6) : 805-824

Bullock, G. L. and Stuckey, H. M., 1975 Fluorescent antibody identification and detection of the *Corynbacterium* causing kidney disease of Salmonids. *J. Fish. Res. Bd. Can.*, **32** : 2224-2227

Bullock, G. L., Stuckey, H. M. and Chen, P. K., 1974 Corynbacterial kidney disease of Salmonids, growth and serological studies on the causative bacterium. *Appl. Microbiol.*, **28** : 811-814

Bullock, G. L., 1976 Identification procedures for the common fish pathogenic bacteria (personal communication)

Chen, P. K., Bullock, G. L., Stuckey, H. M. and Bullock, A. C., 1974 Serological diagnosis of *Corynbacterium* kidney disease of Salmonids. *J. Fish. Res. Bd. Can.*, **31** : 1939-1940

Finlay, J. and Hill, B. J., 1975 The use of complement fixation test for rapid typing of infectious pancreatic necrosis virus. *Aquaculture*, **5** : 305-310

Gratzek, J. B., McGlamery, M. H., Dawe, D. L. and Scott, T., Microcultures of bull-
1973 head (*Ictalurus nebulesus*) cells : Their use in quantification of channel catfish (*Ictalurus punctatus*) virus and antibody. *J. Fish. Res. Bd. Can.*, **30** : 1641-1645

Heartwell, C. M., Immune response and antibody characterization of the channel catfish to
1975 a naturally pathogenic bacterium and virus. *U.S. Fish. Wildl. Serv. Tech*, Pap. No. 85 : 34 pp

Heartwell, C. M. and Panley, G. B., Immune hypersitirity studies in the channel catfish
1975 *(Ictalurus puctatus)*. *J. Fish. Res. Bd. Can.*, **32** : 932-948

Hill B. J., Underwood, B. O., Smale, C. J. and Brown, F., Physico chemical and secro-
1975 logical characterisation of five rhabdovierus infecting fish. *J. Gen. Virol.*, 27 : 369

Jϕrgensen, P. E. V., Serological identification of Egtved virus. *Bull. Offs. int. Epizoot.*, **69** :
1969 985-987

— , Egtved virus : demonstration of neutralizing antibodies in serum
1971 from artificially infected rainbow trout. *J. Fish. Res. Bd. Can.*, **28** : 875-877

— , Egtved virus antigenic variations in 76 virus isolates examined in
1972 neutralizing tests and by means of fluorescent antibody technique (FAT). *In* Symp. Zool. Soc , London ; **30** : 333-340

— , Inactivation of IPN and Egtved virus. *Riv. Ital. Passic. Ittiop.*, **8** :
1973a 107-108

— , The nature and biological activity of IPN virus neutralizing antibodies
1973b normal and immunized rainbow trout (*Salmo gairdnerii*). *Arch. Gesamte Virusforsch.*, **42** : 9-30

— , Indirect fluorescent antibody techniques for demonstrating trout
1974 viruses and corresponding antibody. *Acta. Vet. Scand.*, **15** : 198-205

Jϕrgensen, P. E. V. and Graubelle, P. C., Problems of serological typing of IPN virus
1971 *Ibid.*, **12** : 145-147

Jϕrgensen, P. E. V. and Meyling, A., Egtved virus : demonstration of virus antigen by the
1972 fluorescent antibody technique in tissues of rainbow trout affected by viral haemorrhagic septicaemia and in cell-cultures infected with Egtved virus. *Arch. Gesamte. Virusforsch.*, **36** : 115-122

Klontz. G. W. and Anderson, D. P., Oral immunization of salmonids : a review. *In* 1970 Symp. Diseases of fishes and shell-fishes. Ed. S. F. Snieszko. *Am. Fish. Soc.*, Washington : Spl. Publn. No. 5 : 16-20

Lientz, J. C. and Springer, J. K., Neutralization tests of IPN virus with polyvalent anti- 1973 serum. *J. Wildl. Dis.*, **9** : 120-124

McAllister, P. E., Fryer, J. L. and Pilcher, K. S., An antigenic comparison between in- 1974a fectious haematopoetic necrosis virus (OSU-strain) and viral haemorrhagic septicaemia of rainbow trout (Denmark strain) by cross neutralization. *J. Wildl. Dis.*, **10** : 101-103

McAllister, P. E., Fryer, J, L., Pilcher, K. S., Further characterization of infectious 1974b haematopoetic necrosis virus of salmonid fish (Oregon strain). *Arch. Gesamte. Virusforsch.*, **44** : 270-279

McAllister, P. E. and Pilcher, K. S., Auto-interference in infectious haematopoetic necro- 1974 sis virus of salmonid fish. *Proc. Soc. Exp. Biol. Med.*, **145** : 840-844

McCarthy, D. H., Detection of *Aeromonas salmonicida* antigen in diseased fish tissue. *J.* 1975 *Gen. Microbiol.*, **88** : 384-386

McCarthy, D. H. and Rawle, T. C., The rapid serological diagnosis of fish furunculosis 1975 caused by smooth and rough strains of *Aeromonas salmonicida. Ibid.*, **86** : 185-187

Plumb, J. A., Neurralizations of channel catfish virus by serum of channel catfish. *J.* 1973 *Wildl. Dis.*, **9** : 324-330

Raab, L., Cormick, J. W. and McDermott, L. A., A microscopic slide agglutination test 1964 for the presumptive diagnosis of furunculosis in fish. *Prog. Fish. Cult.*, **26** : 118-120

Ross, A. J. and Klontz, G. W., Oral immunization of rainbow trout against an etio- 1965 logical agent of "red-mouth "disease". *J. Fish. Res. Bd. Can.*, **22** : 713-719

Shotts, E. B. and Snieszko, S. F., Selected bacterial fish diseases. *J. Wildl. Dis.*, **12** : 1976 143-151

Tu, K., Spendilove, R. S. and Goede, R. W., Immunoflurescent cell assay of infectious 1974 pancreatic necrosis virus. *Appl. Microbiol.*, **27** : 593-599

Wolf, K.. Fish Virology : procedures and preparation of materials for plaquing fish viruses 1973a in normal atmosphere. *U.S Fish. Wildl. Serv. Fish. Dis. Leafl.* No. 55 : 1-13

— , Herpesvirus of lower vertebrates. *In* "The Herpesvirus". Ed. A. S. Kapran. 1973b *Acad. Press*, New York : 494-520

Wolf, K. and Quimby, M. C., Fish cell and tissue culture. *In* Fish Physiology Eds. 1969 W. S. Hoar and D. J. Randall, Acad. Press, New York and London ; **3** : 253-305

— , Fish viruses : buffers and methods for plaquing eight agents under normal 1973 atmosphere. *Appl. Microbiol.*, **25** : 659-664

— , Pethijohn, L. L. and Landolt, M. L , Fish viruses : isolation and identification 1974 of infectious haematopoitic necrosis in eastern North America. *J. Fish. Res. Bd. Can.*, **30** : 1625-1627

CHAPTER VI

Infectious protozoan diseases

Infectious protozoan diseases are caused mainly by two parasites *viz.*, *Ceratomyxa shasta* and *Myxosoma cerebralis* ; the former infects the viscera and musculature of the host while the latter parasitizes the branchial cartilage of a fish.

A *Ceratomyxa shasta*

Considerable work on myxosporidians has already been done by Schafer (1968), but very little is known yet about its life cycle and mode of transmission. A more comprehensive account of this parasite has recently been made available by Johnson *et al.* (1978).

1 Geographical distribution

The parasite has a restricted geographical distribution and has not yet been recorded from India. Zinn *et al* (1977) have proved beyond doubt that most salmonid species are susceptible to the disease caused by *C. shasta* and it is no wonder that trouts cultured in India might also be suffering from the attack of this parasite.

2 External and internal signs of infection

External symptoms of *Ceratomyxa* infection are variable ; infected fishes loose appetite, are darker in colour, lethargic and prefer shallow waters. Distended abdomen due to accumulation of ascitic fluid and exophthalmia are the other signs develop later in the disease (Schafer, 1968) ; large pustules filled with fluid containing blood, necrotic tissue, etc., are the other external symptoms (Conrad & Decew, 1966 ; Johnson, 1975).

Internal symptoms of the infection are :

a Mucoid intestinal contents ; posterior intestine swollen and haemorrhagic (Conrad & Decew, 1966) ;
b All layers of hind gut are swollen (Wales & Wolf, 1955) ;

c Abscessed lesions on the musculature (Wood, 1979) ;

3 Identification of the parasite

Noble (1950) described the parasite. The dimensions of the binucleate spores vary from 14-23 μm in length and 6-8 μm in width at the sutural line Smear preparations of intestine and gall bladder and stained by Ziehl-Neelsen method (without heating carbol fuchsin) help in most reliable identification of the parasite (Hoffman and Meyer, 1974).

4 Effect of temperatures on the parasite

At low temperature (below 6.7°C) the infection of *C. shasta* can be suppressed but at higher temperature (between 14 and 23.3°C) mortalities of the fish may occur from the 12th day onwards (Udey *et al* , 1975).

5 Mode of transmission

Direct transmission of *C. shasta* from fish to fish never occurs but salmonids get infected when their ambient waters contain infectious stage of the parasite. Under laboratory codition at 20°C Ceratomyxosis did occur when bottom sediments of a lake, where the disease outbreaks every year, were used (Fryer, 1971). In winter susceptible salmonids could not be infected with the parasite (Johnson, 1975).

6 Prevention and control

No treatment to control the disease is yet known (Needham & Wootten, 1978). Sanders *et al.* (1972) have suggested a combination of MicroFloc filtration follwed by exposure to chlorine (2.2-5.3 ppm for 60 min.) for hatchery management. Ultraviolet radiation has also been found to eradicate the pathogens (Bedell, 1971). Genetic selection and prodution of resistant strains of rainbow and steelhead trouts and chinaok salmon have also been reported against Ceratomyxosis (Johnson, 1975 ; Zinn *et al.*, 1977).

B *Myxosoma cerebralis*

Of all the myxosporidian parasites invading the cartilage of a fish *Myxosoma*

cerebralis is recognized as the most important. *Myxosoma cartilaginis*, encountered from the cartilage at the base of fin rays and gill arches of centrachids (Hoffman *et al.*, 1965), and *Myxobolus aeglefini*, causing erosion or hypertrophy of the cranial cartilages of plaice, hake and haddock (Sindermann, 1970) are found to be less harmful than *M. cerebralis*. As such a bulk of literature is already available on the last named parasite. However, the comprehensive account of *M. cerebralis* has been made available by Halliday (1976).

1 Geographical distribution of *M. cerebralis*

The parasite has been recorded from Africa (Preudhomme, 1970), America (Bogdanova, 1969 ; Margolis, 1972 ; Hnath, 1970 ; Hoffman, 1973 ; Yasutake & Wolf, 1970 ; Tidd and Tubb 1970 ; Hoffman *et al.*, 1962 ; and Hoffman, 1968), Asia (Hoffman, 1970a ; and Sehgal, 1967), Australia, New Zealand (Hewith and Little, 1972) and Europe (FAO, 1972; Luckey, 1970 ; Havelka & Volf, 1970 ; Schaperclaus, 1954a, & b ; Ramussen, 1967 ; Halliday, 1974 ; Ghittino, 1970 ; Uspenskaya, 1955 & 1957 ; Bogdanova, 1968, 1969, and 1970 ; and Tomasec, 1960).

2 External and internal signs of infection

The most conspicuous sign that develops after 2 or 3 months of infection is tail-chasing ; as such, the disease is known commonly as "whirling disease".

Head cartilage, particularly around the auditory capsule, is fed upon by the vegetative forms of the parasite resulting in extensive damage and deformities of the cartilage of trouts. With the advancement of the disease the parasite invades the spinal chord causing severe deformities. The fish looks darker in colour.

3 Identification of the parasite

Morphology of the spores of *M. cerebralis* (Hofer, 1903) was first compared with *M. cartilaginis* (Hoffman, Putz, and Dunbar, 1965) in 1971 (Lom and Hoffman, 1971). The dimensions of spore of the former vary between 7.4. and 9.7 μ in length and 6.2 and 7.4 μ in breadth. The polar capsules 5-6 X 3-3.5 μ. Markiw & Wolf (1974 & 1978) have evolved spore detection methods and recognized *M. cerebralis* using fluorescent antibody techniques. In vitro sporulation of the parasite was done by Wolf & Markiw (1976). Rydlo (1975) quantitatively studied the spores of *M. cerebralis*. Biological

properties of the invasive stage of the parasite have been made available by Uspenskaya (1978). Wolf & Markiw (1979) have used silver nitrate for staining spores and other stages of *M. cerebralis*.

4 Effect of temperature on the parasite

Spores of *M. cerebralis* remain viable even at a very low temperature (−20°C) for a considerable period (Putz, 1970 ; Hoffman & Putz, 1971), but are killed when kept for 10 minutes at a temperature of 60 to 100°C (Hoffman & Putz, 1969).

5 Mode of transmission of the parasite

Transmission of *M. cerebralis* is effected by shipments of live and frozen trouts and their eggs or alevins. Birds and human agencies may help in the transmission of the parasite (Halliday, 1974a).

6 Prevention and control

Schaperclaus (1954), Rasmussen (1965) and Bogdanova (1968) have suggested destruction of the affected trouts to control the infection of *M. cerebralis*. However, disinfection, husbandry, and water treatment have been advocated to prevent "whirling disease" caused by *M. cerebralis* (Rasmussen, 1958, 1961, and 1965 ; Hoffman *et al.*, 1962 ; Brierly & Scott, 1969 ; Hoffman and putz, 1969 ; Ghittino, 1970a ; Hoffman, 1970b & c ; Hoffman and Hoffman, 1972).

Literature cited

Bedell, G. W., 1971 — Eradicating *Ceratomyxa shasta* from infected water by chlorination and ultraviolet irradiation. *Prog. Fish Cult.*, **33** : 51-54

Bogdanova, E. A., 1968 — Modern data on the distribution and biology of *Myxosoma cerebralis* as agent of whirling disease of salmonids. *Bull. Off. Int. Epiz.*, **69** : 1499-1506

— 1969 — , New data on the distribution of *Myxosoma cerebralis* and peculiarities of its ecology, dependig on biotic and abiotic factors. *Prog. Protozool.*, **2** : 265

— 1970 , On the occurrence of whirling disease of salmonids in nature in U.S.S.R. *J. Parasit.*, **56**, *Proc. 2nd Int. Congr.*, Abstr. No. 719

Brierly, R. V. and Scott, E. J., 1969 Fish diseases. A report on their incidence and current therapeutic measures in fish farming. Doc. No. BA R/69/103 ; 60 pp. Wellcome Research Laboratories, Beckenham

Conrad, J. F. and Decew, M., 1966 First report of *Ceratomyxa* in juvenile salmonids in Oregon. *Prog. Fish Cult.*, **28** : 238

F. A. O., 1972 International measures for the control of major communicable fish diseases. E.I.F.A.C. 72/Sc II-6 ; 11 pp

Fryer, J. L., 1971 *Ceratomyxa* in salmonids. *FAO Aquaculture Bull.*, **3** : 12-13

Fryer, J. L., and Sanders, J. E., 1970 Investigation of *Ceratomyxa shasta*, a protozoan parasite of salmonid fish. *J. Parasit.*, **56**, *Proc. 2nd Int. Congr.* Abstr. No. 759

Ghittino, P., 1970a *Piscicoltura e Ittiopatologia*, **2** : 222-232 (Italy)

— 1970b , Present status of whirling disease in Italian trout farms. *Riv. It. Piscic-Ittiopat.*, **5** : 89–92

Halliday, M., 1974a Studies on *Myxosoma cerebralis*, a parasite of salmonids. III Some studies on the epidemiology of *M. cerebralis* in Denmark, Ireland and Scotland. *Nord. Vet. Med.*, **26** : 165-172

— 1974b , Studies on *Myxosoma cerebralis*, a parasite of salmonids. IV A preliminary immunofluorescent investigation of the spores of *M. cerebralis*. *Nord. Vet. Med.*, **26** : 173-179

— 1976 , The biology of *Myxosoma cerebralis* : the causative organism of whirling disease of salmonids. *J. Fish. Biol.*, **9** : 339-357

Havelka, J. and 1970 Volf, F., Whirling disease of salmonids caused by *Myxosoma cerebralis* in Czechoslovakia. *J. Parasit.*, **56** : *Proc. 2nd Int. Congr. Parasit.* Abstr. No. 253

Hewith, G. C. and Little, R. W., 1972 Whirling disease in New Zealand caused by *Myxosoma cerebralis* (Hofer, 1903). *N.Z.J. Mar. freshw. Res.*, **6** : 1-10

Hnath, I. G., 1970 Whirling disease in State of Michigan. *J. Parasit.*, **56** : *Proc. 2nd Int. Congr.* Abstr. No. 273

Hoffman, G. L., 1968 Current status of whirling disease in salmonids in U. S. *Am. Fish. U.S. Trout News* : 10

— 1970a , Intercontinental and transcontinental dissemination and transfaunation of fish parasites with emphasis on whirling disease (*Myxosoma cerebralis*). *Am. Fish. Soc. spec. Publn.*, **5** : 69-81

— 1970b , Whirling disease of trout and salmon caused by *Myxosoma cerebralis* in the United States of America. *Riv. It. Piscic. Ittiopat.*, **5** : 29-31

— 1970c , Effects of disinfecting agents on spores of *Myxosoma cerebralis*. *Prog. Sport Fish Res.* (1970) : 99-100

Hoffman, G. L. and Hoffman, C. L., 1972 Studies on the control of chemicals on spores in *vitro* and of calcium oxide as a disinfectant in simulated ponds. *J. Wildl. Dis.*, **8** : 49-53

Hoffman, G. L. and Meyer, F. P., 1974 Parasites of freshwater fishes : A review of their control and treatment. T. F. H. Publishers, Neptune, N. J.

Hoffman, G. L. and Putz, R. E., 1969 Host susceptibility and the effect of aging, freezing, heat and chemicals on spores of *Myxosoma cerebralis*. *Prog. Fish Cult.*, **31** : 35-37

Hoffman, G. L., Putz, R. E. and Dunbar, C. E., 1965 Studies on *Myxosoma cartilaginis* n. sp. of centrarchid fish and a synopsis of the *Myxosoma* of North American freshwater fish. *J. Protozool.*, **12** : 319-332

Hoffman, G. L. and Putz, R. E., 1970 Bird transmission of whirling disease. *Prog. Sport Fish Res.* (1970) : 100

Johnson, K. A., 1975 Host susceptibility, histopathologic and transmission studies on *Ceratomyxa shasta*, a myxosporidian parasite of salmonid fish. Ph. D. Thesis, Oreg. State Univ. Corvallis, Oreg. : 134 pp

Lom, J. and Hoffman, G. L., 1971 Morphology of the spores of *Myxosoma cerebralis* and *M. cartilaginis*. *J. Parasit.*, **57** : 1302-1308

Lucky, Z., 1970 Pathological changes and diagnostics of myxosporidians of the rainbow trout *(Salmo gairdneri)*. *Acta. Vet. Brno. Suppl.* 1 : 19-29

Margolis, L. and Evelyn, T. P. T., 1975 *Ceratomyxa shasta* disease in chum Salmon *(Onkorhynchus keta)* in British Columbia. *J. Fish. Res. Bd. Can.*, **32** : 1640-1643

Markiw, M. E. and Wolf, K., *Myxosoma cerebralis* : comparative sensitivitv of spore 1974 detection methods. *J. Fish. Res. Bd. Can.*, **31** (10) : 1597-1600

— , *Myxosoma cerebralis* : fluorescent antibody techniques for antigen reco- 1978 gnition. *Ibid.*, 35 (6) : 828-832

Needham, T. and Wootten, R., The parasitology of teleosts. *In* Fish Pathology (Ed. 1978 Ronald J. Roberts). Bailliere Tindall, London : 144-182

Noble, E. R., On a myxosporidian (Protozoan) parasite of California trout. *J. Para-* 1950 *sitol.*, **36** : 457-460

Preudhomme, J. G., Whirling disease of trout in Morocco, *FAO Aquaculture Bull.*, **2** : 14 1970

Putz, R. E., Experimental transmission of *Myxosoma cerebralis* and effect of freezing 1970 on the spores. *Prog. Sport Fish Res.* : 55-57

Rasmussen, C. J., Undersφgelser over effektiviteten of Kalkvaelstof til bekaenpelse of 1958 drejesyge. *Medd. Forsφgsdanbruget*, **9** : 1-2

— , Vejiledning i opdraet of yngel i bassiner, *Ibid.*, **19** : 1-13 1961

— , Whirling disease of rainbow trout and its control in Denmark. *FAO* 1965 *EIFAC*/T_2 : 14-19

— , Handbog i φrredpodrae, 242 pp. : Copenhagen Rhodos 1967

Rydlo, M., Nachweis von Sporen den *Myxosoma cerebralis* in Verschienden Organen 1971 drehkranker Regenbogenforellen. *Zeitschr. ges. Fisch.*, **7** : 97-99

Sanders, J. E., Fryer, J. L., Leith, D. A. and Moore, K. D., Control of the infectious 1972 protozoan *Ceratomyxa shasta* by treating hatchery water supplies. *Prog. Fish Cult.*, **34** (1) : 13-17

Schafer, W. E., Studies on the epizootiology of the myxosporidian *Ceratomyxa shasta* 1968 Noble. *Calif. Fish* and *Game*, **54** : 90-99

Schaperclaus, W., *Fischkrankheiten*. 708 pp. : Berlin Akademic-Verlag. 1954a

— , Undersφgelse of sygdomme hos ovrederne i dankse damburg og forslag 1954b til bekaenpelse heraf. Ferskvands-iskeribla-det, **52** : 145-159

Sehgal, K. L., 1967 Report on the causes of mortality of trout eggs, alevins and fry at Achabal hatchery in 1966-67. Mimeo, 1-13 : *Centr. Inl. Fish Res. Inst.*, Barrackpore, (W. B.), India

Thomson, P. E., 1972 The major communicable fish diseases of Europe and North America : A review of attempt at their control. *EIFAC* 72/ScII Symp., **10** : 35 pp.

Tidd, W. M. and Tubb, R. A., 1970 Investigations of whirling disease in Ohio. *J. Parasit.*, **56** : *Proc. 2nd Int. Congr.*, Abstr. No. 632

Tomasec, I., 1960 Lutte contre les principles maladies infectieuses des poissons. *Bull. Off. Int. Epizoot.*, **54** : 247-252

Udey, L. R., Fryer, J. L. and Pilcher, K. S., 1975 Relation of water temperature to Ceratomyxosis in rainbow trout and coho salmon. *J. Fish. Res. Bd. Can.*, **32** : 1545-1551

Uspenskaya, A, V., 1978 Biological peculiarities of the invasive stage of *Myxosoma cerebralis*. *Parasitologiya*, 22 (1) : 15-19

Wolf, K. and Markiw, *M.* E., 1976 *Myxosoma cerebralis* : in *vitro* sporulation of the myxosporidian of salmonid whirling disease. *J. Protozool.*, 22 (3) : 425-427

— , 1979 *Myxosoma cerebralis* : a method for staining spores and other stages with silver nitrate. *J. Fish. Res. Bd. Can.*, **36** (1) : 13-19

Wood, J. W., 1974 Diseases of Pacific salmon, their prevention and treatment. State of Washington. Deptt. of Fisheries, Hatchery Division, 2nd Edn. : 73 pp.

Yasutake, W. T. and Wolf, H., Occurrence of whirling disease of trout in Western United States. *J. Fish. Res. Bd. Can.*, **27** (5) : 955-956

Zinn, J. L., Johnson, K. A., Sanders, D. E. and Fryer, J. L., Susceptibility of salmonid species and hatchery strains of chinook salmon to infections by *Ceratomyxa shasta*. *Ibid.*, **34** : 933-936

—