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Disease Outbreaks in the Shrimp Culture Grow-out Systems of Sri Lanka.

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ABSTRACT. Disease outbreaks in shrimp culture systems of Sri Lanka has resulted in a 35 to 70% reduction in production at farm sites. Several critical water quality parameters (Salinity: 2-31 ppt; sulphides: 0.24-0.67 mg/l; Nitrites: 09-36 mg/l; total suspended solids: 80-160 mg/l) in the main water source (Dutch canal) were at sub-optimal ranges during disease outbreaks.

Microfouling on shells, reduced moulting, black gill syndrome were the most commonly recorded symptoms. Infestations by Monodon Baculovirus (MBV) and peritrichous ectocommensal Zoothamnium sp. were common in affected farms.

Accumulation of iron in-between gill lamellae and on lamellar cuticle was confirmed by Scanning Electron Micrography (SEM), Transmission Electron Micrography (TEM) and by histochemical studies. Several histo-pathological changes were observed in gill, hepatopancriatic and myocardial tissues.

INTRODUCTION

Shrimp culture in Sri Lanka commenced in the mid 1980's in the coastal belt of the north western province. The areas under shrimp farming and the foreign exchange earnings from the industry have increased considerably during the past few years. Around 1200 ha of land have been allocated for shrimp farming. According to the National Aquatic Resources Agency (NARA), medium and large scale farms have increased to 71, and 160 small scale farms which are usually under 1 ha in extent are operational.

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Shrimp exports have contributed 53% to 73% of the total foreign exchange earnings from the fisheries sector during the period 1985 to 1992 and foreign exchange earnings amount to Rs. 571 million. According to the 5 year plan of the Ministry of Fisheries and Aquatic resources the targeted foreign exchange earnings from the cultured shrimps is Rs. 1252 million in year 1994 (Ministry of Fisheries and Aquatic Resources, 1991).

Shrimp culture systems of Sri Lanka are operated at semi-intensive and intensive levels (Jayasinghe, 1991). Estuaries and lagoons are the main sources of water for farm operations. The dutch canal serves as the water source as well as the outlet for discharged water for more than 60% of the existing farms. Several lands with acid sulphate and potential acid sulphate soils have been converted to shrimp farms in this area.

Gills are the primary site of respiration and osmoregulation in shrimps (Robertson, 1960). Gills are highly sensitive to stress and are susceptible to diseases and parasitic infections which pose serious problems in shrimp culture systems (Foster and House, 1978).

The hepatopancreas is a vital and major organ involved in diverse metabolic activities. It is primarily responsible for the synthesis and secretion of digestive enzymes and subsequent uptake of nutrient materials. Other major functions of the hepatopancreas include excretion, storage of organic reserves, and lipid and carbohydrate metabolism (Gibson and Barker, 1979; Storch and Lehnert-Moritz, 1980). Infections by virus, bacteria and by rickettsial organisms are reflected in this organ.

The heart tissues are also vulnerable to infestations by pathogens (Couch, 1978, Lightner, 1988). In addition, hypoxia induced changes can be identified in heart tissues (Nash *et al.*, 1988; Nash, 1990).

This paper reports the results of a study on histopathology of cultured shrimp during 1989/1990 disease outbreaks.

MATERIALS AND METHODS

Weekly sampling of shrimps for histo-pathology, were carried out at selected commercial farms, bordering the Dutch canal as a part of an indepth study to investigate disease outbreaks in relation to soil and water quality during 1989/1990. From each farm site randomly selected samples of 10-15 shrimps were sacrificed by injecting Davidson's fluid. Gills, hepatopancreas and hearts were removed for pathological studies. For light microscopic studies (LM), tissues were stained with either haemotoxylin and eosine (H and E) or with Perl's Prussian blue for the demonstration of iron. Symptoms observed during sampling were noted. Production data during and before disease outbreaks were obtained through interviews with farmers. In a parallel study, water quality parameters of Dutch canal and detail investigation on soil acidity, potential acidity, were carried out (Jayasinghe, 1991).

Gill samples were processed for scanning and transmission electron microscopic studies. A Phillips PSM 500 Scanning electron microscope and a Phillips 301 electron microscope were used in these studies.

RESULTS AND DISCUSSION

The accepted ranges, optimal levels and values recorded at the Dutch canal of important - water quality parameters for shrimp culture are given in Table 1.

Microfouling on shell, dying on pond sides, black spot, reduced feeding, black/brown gill condition, tail rot, black spot, reduced growth, soft shell condition and red/brown deposits on belly were the common symptoms in shrimps recorded in the present study. Table 2 presents the most widely recorded symptom and the production before and during disease outbreaks at selected eight farm sites.

Scanning micrograph of the gills collected from disease specimens is given in Plate 1. Gills of some affected shrimps were found to be covered with a heavy layer of coarsely granular material in SEM studies. Detritus was also found trapped in these deposits and the lamellae were swollen. Histochemical studies on the above gills using Prussian blue stain demonstrated the presence of iron (Plate 2). Concurrent TEM sections (Plate 3) very clearly indicate the electron dense material.

Table 1. **Accepted ranges of water quality, optimal levels for shrimp culture, and levels recorded in Dutch canal during disease outbreaks (Boyd, 1989; Poernomo, 1990).**

Parameter	Acceptable range	Optimal* level	Values recorded in Dutch canal
Temperature °C	26-33	29-30	29-32
Turbidity (FTU)	0-150	2-30	16-32
Total suspended solid (mg/l)	2-14	<5	80-160
Dissolved oxygen level (mg/l)	3-12	4-7	4.0-7.2
Salinity (ppt)	10-35	15-20	02-31
pH	7.5-8.7	8.0-8.5	7.6-8.5
Nitrate Conc (mg/l)	0-200	na	0.001-0.065
Nitrite Conc (mg/l)	<0.25	<0.02	0.091-0.360
Ammonia conc (unionised mg/l)	<0.25	<0.11	0.23-0.37
Hydrogen sulphide conc (mg/l)	<0.25	<0.002	0.24-0.67
BOD_5 (mg/l)	<10	na	8-54

na - not available

Table 2. Shrimp production before and during disease outbreaks, symptoms observed and % reduction in production due to disease outbreaks.

Farm	Production before disease outbreak mt/ha/cycle	Production during disease outbreak mt/ha/cycle	Symptoms observed	Reduction in production
A	3.3	0.9	1,2,3,4,8	12%
B	1.9	1.03	4	45%
C	6.7	1.8	2,4,5,6,8,13	73%
D	7.5	3.5	1,2,3,8	53%
E	10.5	3.25	4	69%
F	1.9	0.9	4,13	52.6%
G	0.88	0.57	1,2,3,8,10	35.2%
H	9.6	3.2	1,2,3,11,10	66%

Key:

1	microfouling on shells	8	dying on pond sides
2	reduced frequency of molting	9	size disparity
3	reduced feeding	10	empty guts
4	black gills	11	red/brown deposit on belly
5	soft shell condition	12	shrimp with red coloration
6	tail rot	13	reduced growth
7	black spot		

Source: ADB 1990; Jayasinghe 1991

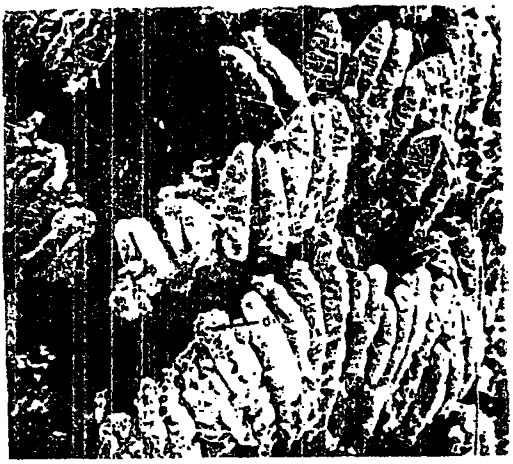

Plate 1. **Scanning electron micrograph of a gill with deposits (d) on gill lamellae x 600.**

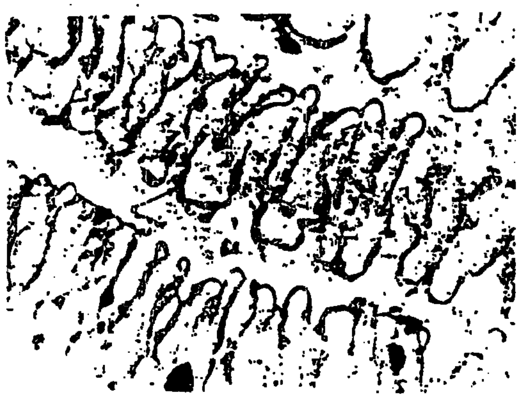

Plate 2. **Iron containing deposits (d) stained with Pearl's prussian blue in gills of shrimp (L.S. of gill) x 1700.**

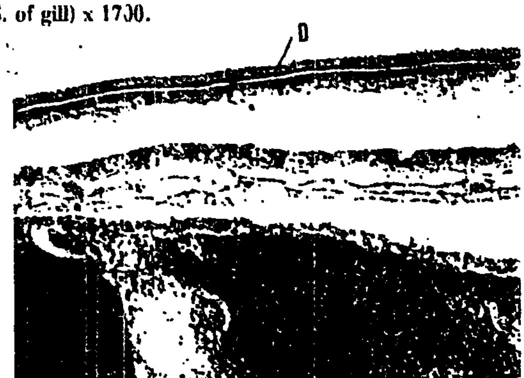

Plate 3. **Transmission electron micrograph of a lamellar cuticle of a shrimp showing electron dense deposits (D) X 14700.**

Affected shrimp were frequently found infested with ectocommensal protozoans in LM studies. These ciliated protozoans were found attached to the tips of the gill filaments by a stalk. The SEM studies on the gill samples showed the detailed structure of the organism and the mode of attachment of the ectocommensal to the host gill (Plate 4). The base of each stalk terminated in a circular disc that is attached to the shrimps gill cuticle. No mechanical damage could be observed.

LM studies of the H&E stained histological sections of gills revealed structural changes in the gill lamellae. Lamellae were swollen and the epicuticular layer was found separated in brown-gill shrimps with increased vacuolations and dilated blood sinuses in some animals.

All the cell types described by Staniar *et al.*, (1986); Gibson and Baker (1979); Dall and Moriarty (1983) were well represented in the tubules of the hepatopancreas of the healthy shrimps (Plate 5a). Considerable areas of the tubules were occupied by very clear, well vacuolated R-cells (Restzellan). Darkly stained F-cells (Fibrillenzellan) and B-cells (Blasenzellan) were observed in normal shrimps. The lumens of the tubules were filled with secretions.

The hepatopancriatic tubules of the affected shrimps were dominated by B-cells (Plate 5b). R-cells were very poorly represented. The lumens of the tubules were without secretions. The F-cells were not observed frequently. It may also to be noted that signs of parasitic infestations were observed in hepatopancreatic tissues of the shrimps. Haemocyte infiltrations and hemocytic encapsulations were observed in haemosinuses of tubules. Chromatin marginated, hypertrophid nucleii were also observed. MBV occlusion bodies were found in most of the shrimps. The nuclei of the myocardial cells and the nucleii of associated satellite cells are very clear in the heart tissue of normal shrimps as described by Bell and Lightner, (1988).

Necrotic areas in the myocardium were observed in affected shrimps. Myocardial fibres appeared split and fragmented into multiple rounded, shrunken masses of multivacuolated tissue. The nuclei of the fragmented areas showed evidence of disintegration. Haemocytic infiltrations and encapsulations and even nodule formations were noted near necrotic areas indicating signs of severe infections.

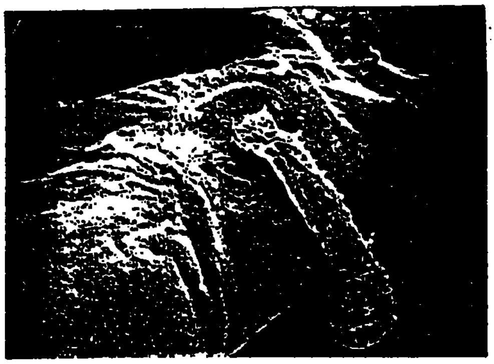

Plate 4. Scanning electron micrograph of *Zoothaminium* mode attachment to gill filament of the shrimp Zooid (Z), stalk (S), basal disc (b) and gill lamellae (L) X 3500.

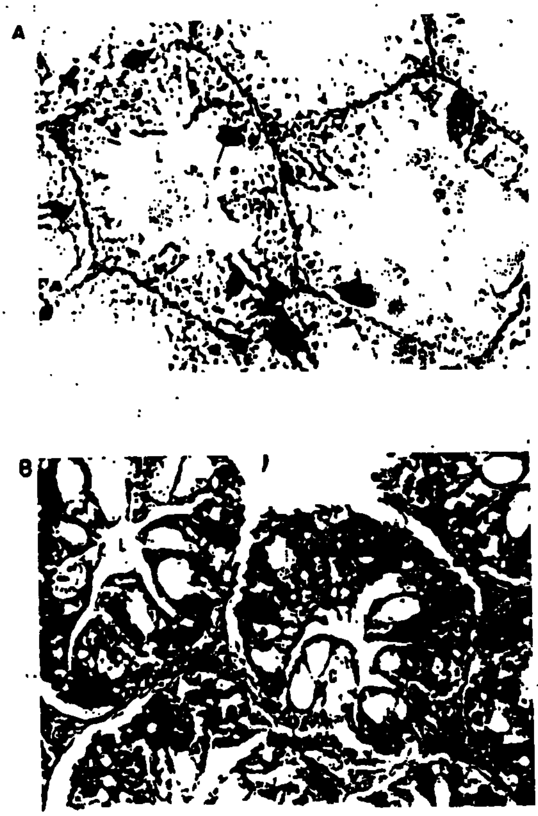

Plate 5. Transverse sections of a hepatopancreatic tubule of (A) healthy shrimp, B affected shrimp. B-cells (C), f-cells (F) lumen (L). (H & E X 450).

Stress generated through poor water quality, poor soil quality and deteriorating environmental conditions are most likely to provoke histological changes and disease outbreaks resulting in poor growth and low survival in shrimp culture systems (Lightner, 1988; Sindermann, 1989; Liao, 1989; Nash, 1990). The variations in the basic water quality parameters such as pH, and dissolved oxygen concentrations recorded in the present study were within the acceptable ranges specified by Apud *et. al.*, (1989), Chiu (1988 b), Boyd (1989) and Poernomo (1990) for shrimp culture systems. Results also revealed that the ranges of nitrites, sulphides and suspended solids were not within the recognised accepted limits (Wickins, 1981; Chiu, 1988 a & b; Boyd, 1989) in Dutch canal during disease outbreak.

Brown gill colour in gills appears to be one of the main symptoms recorded (Table 2). Brown gill syndrome has been widely reported in cultured as well as in wild crustaceans (Rinaldo and Yevich, 1974; Lightner and Redman, 1977; Greig *et al.*, 1982; Lightner, 1988; Sindermann, 1989 and Nash *et al.*, 1988). Environmental degradation (Sawyer, 1982; Sindermann, 1989); infestations with epiphytic and epizoic organisms (Couch, 1978; Lightner, 1988; Sindermann, 1989); exposure to heavy metals (Couch, 1978; Greig, *et al.*, 1982) and accumulation of iron-containing deposits (Nash *et al.*, 1988; Nash, 1990, Jayasinghe 1991) have been related to the appearance of brown gills. Lightner (1988); Nash *et al.*, (1988); and Nash (1990) have also indicated the presence of iron deposits among gill lamellae or encrustations on gill lamellae of shrimps cultured on acid sulphate soils or acidic culture systems rich in iron.

In a parallel study (Jayasinghe, 1991), the possibility of formation of iron hydroxides and manganese hydroxide at the bottom of shrimp culture ponds where disease specimens were collected has been clearly demonstrated. Highly pyritic soils of these ponds exhibit acid sulphate /potential acid sulphate conditions (Jayasinghe 1991). There is a tendency for these hydroxides to enter into the gills of the shrimps with respiratory currents (Jayasinghe, 1991).

The fine structure of the ectocommensals closely reassembled the peritrichous ectocommensal of the genus *Zoothamnium* as described by Johnson *et al.* (1973), Overstreet (1973), Johnson (1976) and Foster *et al.* (1978).

According to Foster *et al.* (1978), Lightner and Redman (1977), and Couch (1978), *Zoothamnium* sp. have a peritrichus mode of attachment.

Here the ectocommensals cause no mechanical damage to the underlying tissue and elicit little response by the shrimp's haemocytes.

The histology and the function of various cell types of the hepatopancreatic tubules are well documented (Stainer *et al.*, 1986); Hopkin and Nott, 1980; Dall and Moriarty, 1983). B-cells are characterized by a large single vacuole containing digestive enzymes. The F-cells synthesize digestive enzymes which accumulate in vacuoles that enlarge by pinocytosis of nutrients and fluids from the tubular lumen. Fatty acids, neutral lipids and phosphor lipids (Momin and Rangneker, 1975) and lipid droplets have been identified in R-cells. According to Lozzi and Peterson (1971) and Momin and Rangneker (1975), R-cells are the major site of lipid absorption, digestion and lipid metabolism. These cells also metabolize and store glycogen.

Hopkins and Nott (1980) and Dall and Moriarty (1983) indicate that B-cells can presumably develop from the F-cells in the fasting animals, when no luminal nutrients are present for absorption and development. R-cell activity is an indication of active lipid absorption, digestion and lipid metabolism in shrimps (Dall and Moriarty, 1983). The predominance of B-cells and the lack of R-cell activity in the tubules of affected shrimps indicate that they are in a poor nutritional state in spite of receiving the normal recommended ration of supplementary feed. There were severe infections in heaptopancreatic tissues in dying shrimp in the present study.

The chromatin marginated hypertrophied nucleii observed in the hepatopancreas are per-occlusion changes of viral infection. MBV (Monodon Baculo virus) occlusion bodies confirmed the MBV infections in the present study. Histological changes related to depleted oxygen supply to myocardial tissue and evidence of severe infestations with bacteria were observed in affected shrimps. The iron containing deposits observed among gill lamellae can obstruct the normal respiratory current affecting the normal gas exchange *via* the respiratory surface leading to the above changes.

CONCLUSION

In conclusion, the adverse water quality in the Dutch canal which serves as the water source and the adverse soils conditions related to acid sulphate appears to be indirectly responsible for investigated disease outbreaks.

Rehabilitation of the water source to improve the water quality, incorporation of an effluent treatment system to reduce suspended solid concentration in the water, amelioration procedures to prevent formation of iron hydroxides in the pond environment can contribute significantly to reduce the risk of disease outbreaks.

REFERENCES

ADB, (1990) Sri Lanka appraisal report on present disease situation in shrimp farms of Sri Lanka. Ministry of Fisheries and Aquatic Resources, Colombo. pp 55.

Apud, F.D., Primavara, J.A. and Torres, P.I. (1989). Farming of prawns and shrimps. Aquaculture extension manual, no. 5. Third edition. SEAFDEC, Iloiilo, Philippines, 65 pp.

Bell, T.A., and Lightner, D.V. (1988). Hand Book of Normal Penaeid Shrimp. Histology World Aquaculture Society Publication, Allen Press Inc. Kansas USA.

Boyd, C.E. (1989). Water quality management and aeration in shrimp farming. Fisheries and Allied Aquaculture Series no. 2. Aubrn University, Alabama. 79 pp.

Chiu, Y.N. (1988a). Site selection for intensive prawn farms. pp 25-26. *In*: Chiu, Y.N., Santos, L.M. and Juliano, R.O. (Eds). Technical Considerations for the Management and Operation of Intensive Prawn Farms. Up Aquaculture Society. Philippines.

Chiu, Y.N. (1988b). Water quality management for intensive prawn ponds. pp 102-128. *In*: Santos, Y.N. and Juliano, R.O. (Eds). Technical Considerations for the and Operation of Intensive Prawn Farms. Up Aquaculture Society. Philippines.

Couch, J.A. (1978). Diseases, parasites and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and Atlantic coast of North America. Fishery Bulletin. 76: 1-44.

Dall, W. and Moriarty, D.J.W. (1983). Functional aspects of nutrition and digestion. pp 215-251. *In*: Mantel, H.L. (Ed). The Biology of Crustacea. vol. 5. Academic Press. New York.

Foster, C.A. and House, H.D. (1978). A morphological study on gills of the brown shrimp. Tissue and cell. 10(1): 77-92.

Foster, C.A., Sarhie, T.G. and Hawkins, W.E. (1978). Fine structure of the perittrichous ectocommensal *Zoothamnium spp.* with emphasis on its mode of attachment to penaeid shrimp. Journal of Fish Diseases. 1: 321-335.

Greig, R.A., Sawyer, T.K., Lewis, E.J. and Galasso, M.E. (1982). A study of metal concentration in relation to gill colour and pathology in the rock crab. Arch. Environm. Contam. Toxcol. 11: 539-545.

Gibson, R. and Baker, P.L. (1979). The decapod hepatopancreas. Oceanogr. Mar. Biol. Ann. Rev. 17. 285-346.

Hopkins, S.P. and Nott, J.A. (1980). Studies on the digestive cycle of the shore crab *Carcinus maenas* (L) J. Mar. Biol. Assoc. U.K. 60. 891-907.

Jayasinghe, J.M.P.K. (1991). The utilization of acid sulphate soils for shrimp culture on the West coast of Sri Lanka. Ph.D. thesis University of Stirling. U.K.

Johnson, S.K. (1976). Chemical control of peritrichous ciliates on young penaeid shrimps. Leaflet No. FDDL 57. 5 pp. Texas A & M University, USA.

Johnson, S.K., Parker, J.C. and Holcomb, W.H. (1973). Control of *Zoothamnium* on penaeid shrimp. Proc. World Mariculture Soc. 4: 321-331.

Liao, I.C. (1989). Penaeus monodon culture in Taiwan, Through two decades of growth. Int. J. Aq. Fish Tecnol. 1: 16-24.

Lightner, D.V. and Redman, R.M. (1977). Histochemical demonstration of melanin in cellular inflammatory processes of penaied shrimp. J. Invert. pathol. 30: 298-302.

Lightner, D.V. (1988). Crustacean Diseases. pp. 8-135. *In:* Sinderman C.J. and Lightner D.V. (Eds). Disease diagnosis and control in North American aquaculture. Elsevier Scientific Publishing Company. Amsterdam.

Lozzi, R.F. and Peterson, D.J. (1971). Lipolytic sites in crayfish hepatopancreas and correlation with fine structure. Comp. Biochem, Physiol. B 39 : 227-236.

Momin, M.A. and Rangneker, P.V. (1975). Histochemical localization of oxidative enzymes in the hepatopancreas of *Scylla serrata* J. Exp. Mar. Biol. Ecol. 20:249-264.

Ministry of Fisheries and Aquatic Resources. (1991) Five Year Plan. Colombo. Sri Lanka. 25 pp.

Nash, G., Anderson, I.G. and Shariff. M. (1988). Pathological changes in the tiger prawn *Penaeus monodon*. Fabricius, associated with culture in brackish water ponds developed from potentially acid sulphate soils. Journal of Fish Diseases, 11:113-123.

Nash, G. (1990). *Penaeus monodon* grow-out diseases. Paper presented at Aquatec 90. Putra World Trade Center, Kualampoure. 35 pp.

Overstreet, R.M. (1973). Parasites of some Penaeid Shrimps with emphasis on reared hosts. Aquaculture, 2:105 - 140.

Poernomo, A.T. (1990). Site selection for coastal shrimp ponds. Proc. Conference "Shrimp 90" Malaysia.

Rinaldo, R.G. and Yevich. P. (1974). Black spot gill syndrome of the northern shrimp *Pendulous borealis*. Journal of Invertebrate pathology. 24: 224-233.

Robertson, J.D. (1960). Osmotic and ionic regulation. *In.* Waterman (Ed). The Physiology of Crustacea; Academic Press Inc. New York.

Sawyer, T.K. (1982). Distribution and seasonal incidence of black gill in the rock crab, *Cancer irroratus*. pp 199-211. *In*: G.F. Mayer (Editor). Ecological stress and the New York Bight: Science and Management. Estuarine Research Federation, Colombia, South Carolina.

Sindermann, C.J. (1989). Principle Deceases of Marine Fish and Shell Fish., (Volume 2). Academic Press Limited, London. 502 pp.

Stanier, J.F. Woodhouse, M.A. and Grifitin. R.L. (1986). The fine structure of the hepatopancreas of *Carcinus maenas* Crustaceana 14: 56-66.

Storch, V and Lehnert-Moritz. K. (1980). The effects of starvation on the hepatopancreas of the isopod *Ligia*. Zool Anz. Jena., 204: 137-146.

Wickins, J.F. (1981). Water quality requirements for intensive aquaculture. A review. Proc. World. Symp on Aquaculture., Stavanger. 1:17-37.