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GULF COAST REGIONAL SYMPOSIUM ON DISEASES OF AQUATIC ANIMALS

Editors

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LSU-SG-74-05

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PROCEEDINGS OF GULF COAST REGIONAL SYMPOSIUM ON DISEASES OF AQUATIC ANIMALS

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FORWARD

Symposium Goals

The objectives of this symposium were:

1) to bring together those individuals in the Gulf Coast region who were interested in diseases of aquatic animals,
2) to determine the present status of information on disease problems in the Gulf Coast area,
3) to assess future areas of investigation in disease problems, and
4) to promote communication and exchange of information.

Acknowledgments

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AQUATIC ANIMAL DISEASES: SOME CRITICAL ISSUES IN 1974

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INTRODUCTION

Aquatic animal disease problems are becoming recognized as significant, even as major deterrents to successful production operations. This is particularly true of disease problems in marine aquaculture since the importance of disease control in fresh-water trout and salmon hatcheries has been recognized for many decades, and effective measures have been instituted. There is no implication, however, that disease problems no longer exist in fresh-water hatcheries -- in fact, they seem to be increasing because of newly-recognized pathogens, principally viruses.

Disease problems have appeared and, in some cases, persisted in production of other aquatic animals, vertebrate and invertebrate. Natural populations of aquatic animals are also subject to mass mortalities, some of which are caused by epizootic disease.

Of the many issues of importance and interest in aquatic animal disease research, this paper will consider the following:

1) The role of disease as a limiting factor in aquaculture;

2) The definition of environmental stress as a major determinant of aquatic animal disease;

3) Problems created by introductions and transfer of aquatic animals; and

4) The relation of aquatic animal disease to human disease.

DISEASE AS A DETERRENT TO AQUACULTURE

Two years ago, at the Third Annual Workshop of the World Mariculture Society, disease problems were characterized as "ghosts, dragons, or Rumpelstiltskins." To an ever-diminishing number of aquaculturists, disease is still a ghost since they have been spared a visitation by mass-mortalities due to specific pathogens. To others who have been so visited, disease often rears up as a dragon, as when it eliminates scarce brood stock or a larval or post-larval population almost overnight, even when other environmental factors seem reasonably adequate. To still others, probably a majority, disease is a Rumpelstiltskin -- working diligently in the background and chewing away at the number of individuals, especially larvae, post-larvae, and juveniles in tanks, ponds, or raceways.

Dollar values for losses sustained because of disease and the necessity for disease control have been estimated for trout and shrimp production at from 20-30 percent of total costs. There is little residual doubt about their importance, even though such estimates may be inflated by inclusion of unexplained mortalities arbitrarily attributed to disease but really caused by lethal levels of other environmental factors.

At present there are two emerging disease problems in aquaculture (among the many continuing problems which exist and which will be considered later in this symposium). These are virus diseases and the special case of larval diseases.

Viruses of aquatic vertebrates and invertebrates have received much recent attention. Three years ago, only one virus disease of marine invertebrates was known, that attacking crabs. Since then, two quite distinct virus diseases have been reported for oysters, two more for crabs, two for cephalopods, and one for shrimps. One of the oyster viruses was implicated in higher mortality rates in a very limited study, while the shrimp virus was observed in shrimps being used experimentally in studies of effects of chlorinated hydrocarbons. Unfortunately, no established cell lines from marine invertebrates are presently available to carry on adequate studies of the organisms already recognized.

Much current emphasis is focused on virus diseases of freshwater fishes. At a 1972 FAO-sponsored symposium on communicable diseases of fish, about 80 percent of the time and discussion was

concerned with viruses, producing an impressive array of present-day problems:

1) Viral Hemorrhagic Septicemia (VHS) of trout in Europe, but not in United States;

2) Infectious Hematopoetic Necrosis (IHN) of rainbow trout and salmon in United States, but not in Europe;

3) Ulcerative Dermal Necrosis (UDN) of salmon in western Europe, especially in the British Isles;

4) Infectious Dropsy Complex of carp in Europe, in which a dominant viral role is emerging; and

5) Swim Bladder Disease of carp, which moved westward out of Russia and reached West Germany a few years ago. It is presumed to be of viral etiology.

Furunculosis, lymphocystis, and whirling disease of salmonids were also considered, but the relatively recently recognized viral infections clearly occupied center stage.

Larval diseases in aquaculture operations are extremely difficult to cope with -- beyond the ultimate and often undesirable solution of discarding the entire batch. Larvae are very small and their numbers are large, so the individual organisms do not receive proper scrutiny. Only when mortalities reach unusual levels is any attention paid to the possible role of disease in larvae. Reasonable levels of Fred Meyer's "Arkansas Dwindles" are tolerated, as long as larval populations do not dwindle too much or too rapidly. Several recent reports describe diseases of larvae: the fungus Lagenidium in shrimp larvae; the bacterium Leucothoix in lobsters; and an unidentified fungus disease of Macrobrachium larvae. Again though, at least some of these larval diseases are undoubtedly indications of other environmental problems, especially nutrition and water quality, and are produced by secondary pathogens.

DISEASE AND ENVIRONMENTAL STRESS

One of the really exciting, extremely complex, and often very frustrating aspects of present-day aquatic animal disease research is that concerned with environmental influences -- the examination and definition of environmental stress as a major determinant of disease. It has been long recognized that non-optimum conditions in culture operations, such as high temperatures, low oxygen, inadequate diets, and presence of metabolites in closed systems, could enhance effects of known pathogens and encourage activities

of facultative pathogens.

In considering such problems in aquaculture, it is important to distinguish between what we can label primary pathogens, such as Gaffkya (Pediococcus) in lobsters, which can kill even when other environmental factors are reasonably adequate, and facultative or opportunistic pathogens such as vibrios, pseudomonads, and aeromonads which kill when other physiological or environmental factors are poor or marginal. What is referred to as disease in culture operations is often a consequence of one or more of such marginal environmental factors: nutrition, water quality, oxygen, temperature, salinity, and high bacterial populations.

More recently, some effects of other environmental stresses have been recognized -- particularly those resulting from the degrading of natural waters by humans. The association of aquatic animal disease with pollution is still somewhat tenuous but is becoming more definitive.

Dr. Snieszko of the Bureau of Sport Fisheries and Wildlife, in an excellent article just published in the Journal of Fish Biology (Snieszko, 1974) summarizes present knowledge of the relationships between environmental stress (including various kinds of pollutants) and fish diseases. One association which he mentions, and which should be further emphasized, is the emerging information about pollution-enhanced increases -- even population explosions -- of bacteria of the Vibrio-Pseudomonas-Aeromonas groups. Suggestions of these increases exist in reports from marine as well as fresh water. High population densities of such heterotrophic and potentially pathogenic microorganisms, caused in part by high levels of organic material in the aquatic environment, can increase infection pressure on aquatic animals of all kinds, animals which are often being subjected simultaneously to other respiratory and chemical stresses in degraded environments.

One of the best recent examples of this stress complex was the outbreak of mortalities in fish, amphibians, and even reptiles in Lake Apopka, Florida, three years ago, due principally to Aeromonas liquifaciens (Shotts et al., 1972). The lake had been heavily polluted by sewage and citrus processing waste, and the populations of aeromonads were very high.

Another example concerns a non-specific condition known as "fin rot" in fish (Fig. 1) which seems associated with degradation of the coastal marine environment. This should not be considered as an illustration of completed research, but rather as an example of the complexity of problems that emerge once the environment intrudes on the otherwise peaceful, ordered existence of the pathologist.

Recent quantitative surveys along the Middle Atlantic coast have disclosed high prevalence (up to 38 percent) of "fin rot" in samples of trawled marine fishes from the New York Bight. Thus far, 22 species have been found to be affected, and bacteria of the genera Vibrio, Aeromonas, and Pseudomonas were most frequently isolated from abnormal fish. An association between high fin rot prevalences and high coliform counts in sediments is emerging (Mahoney et al., 1973), as is an association between high fin rot prevalence and high heavy metal levels in sediments (Carmody et al., 1973). The disease signs can be reproduced experimentally by exposure of fish to polluted sediments or to bacterial isolates from abnormal fish. Invertebrates such as lobsters and crabs from grossly polluted areas of the New York Bight are also abnormal, with appendage and gill erosion a most common sign. Similar evidence of fish abnormalities possibly associated with degradation of coastal waters has been reported from California (Young, 1964; Southern California Coastal Water Research Project, 1973), Florida (Sindermann, unpublished observations), and the Irish Sea (Perkins et al., 1972).

These few insights strongly suggest more extensive and intensive investigations -- and here we need broadly trained biologists, chemists, microbiologists, and ecologists to work with the pathologist -- to provide information about pollutant effects on enzyme systems, pollutant effects on immunity responses, chemical effects of pollutants on mucus, synergistic effects of pathogens and contaminants, and transfer of resistance factors in bacteria.

A multidisciplinary team is obviously required. Large infusions of money and technical competence will be necessary to explore all aspects of the problems.

PROBLEMS CREATED BY TRANSFERS AND INTRODUCTIONS OF FISH AND SHELLFISH

Justifiable concern has been expressed about possible serious consequences of introductions of infectious disease of aquatic animals into areas otherwise free of the diseases. Examples of the spread of such diseases in this way can be found; the whirling disease of salmonids in the United States, caused by the protozoan Myxosoma cerebralis, is probably the best example.

A number of actions are being taken or will soon be taken to confront the problem. An event important to fish pathologists took place May 1, 1974, when FAO convened the eighth session of the European Inland Fisheries Advisory Commission (EIFAC) in Scotland. A proposal was made by FAO for a convention on international control of major communicable fish diseases -- this emerging from a symposium on that topic held in Amsterdam in 1972. Some of the

wording of the proposal is worth noting here, since it could drastically increase the work-load of fish pathologists in this country as well as elsewhere in the world:

> The spread of communicable fish diseases through international trade in live fish and fish eggs presents considerable dangers of economic and ecological damage. These dangers have induced some countries to ban or consider banning all imports of live fish and fish eggs. Yet some international traffic in live fish and fish eggs is recognized as desirable to improve or supplement local stocks, to meet specific management needs and for genetic improvement in fish culture. EIFAC recognized the urgent need for control over this international traffic and concluded that international control measures, to be practicable and effective, should be based upon inspection for communicable diseases and appropriate certification of live fish and fish eggs at source before shipment.
>
> It was also concluded that sufficient agreement existed in the scientific community on the scientific bases for international control measures, and that such measures should be based on an international convention covering the following points:
>
> 1) acceptance of the principles of certification based on inspection at source and of a uniform certificate for live fish and fish eggs;
>
> 2) the designation by contracting states of national authorities responsible for inspection, certification and control; and
>
> 3) the specification of minimum standards of qualification for national certifying officers and standard methods of detection and analysis of diseases.

Clear and demonstrated pathogens (such as IPN, Myxosoma, VHS) which appear to exist only in certain geographic areas should be subjects of stringent legislation; there is less urgent need for import regulation of diseases which are already widespread.

Here in the United States, three pieces of legislation, in various stages of drafting, hearings, or revision, should be of interest to aquatic animal disease specialists. Provisions of the Lacey Act, designed to prevent introduction of dangerous or harmful animals, are being broadened. The Sullivan Bill, specifying diseases which will exclude certain species from importation or interstate movement, is being drafted. The bill provides for training of inspectors and indemnification for seizure. Also, an Aquaculture Bill has been introduced. Among other provisions, it specifies research on diseases of aquaculture animals.

RELATION OF AQUATIC ANIMAL DISEASE TO HUMAN DISEASE

Whenever the subject of disease in aquatic animals is discussed, the average person seems interested primarily in its possible effect on humans. The distinction is not always made clear to such people between diseases of aquatic animals and those few human diseases which may be transmitted passively by eating raw aquatic animals -- or those very few disease agents of aquatic animals which may also produce disease in humans. There are three general categories of relationships:

1) Aquatic animals may become infected with many pathogens and parasites which are not transmissible to humans (this includes the vast majority of aquatic animal diseases).

2) Aquatic animals may become infected by a few pathogens and parasites which are transmissible to humans. For example, fish and shellfish can become infected with Vibrio parahaemolyticus -- some strains of which can produce enteric disorders in humans if infected animals are eaten raw or undercooked. Six outbreaks of foodborne illnesses due to V. parahaemolyticus and traceable to shellfish were reported by the Public Health Service in 1972. (Of course an outbreak is defined as an incident in which as few as two people experience a similar illness after ingesting a common food.)

As another example, marine fish, especially herring-like fish, can be infected with larval anisakid nematodes (subfamily Anisakinae) which can cause severe allergic inflammatory responses in the human digestive tract if ingested alive (Fig. 2).

3) Fish and shellfish may ingest and accumulate (or transmit passively) microorganisms and toxic substances harmful to humans but relatively harmless to the animals. Included here would be microbial pathogens causing hepatitis, typhoid, and cholera. (The Naples outbreak of the summer of 1973 which killed an estimated 26 people was blamed in part, at least, on contaminated mussels eaten raw.) Also included would be biotoxins such as paralytic shellfish toxin derived from algae and toxic effects of accumulated heavy metals and chlorinated hydrocarbons.

Mercury poisoning of the human population near Minamata Bay in Japan from 1953 to 1961 because of contaminated seafood is probably the most extreme example of the risks involved in eating seafood from badly contaminated waters. We have had no comparable extreme example in the United States, nor do we have as yet any evidence of acute human disease resulting from consumption of fish with high chlorinated hydrocarbon levels.

Possible links between aquatic animal disease and human disease should not be dismissed lightly, nor should they cause panic-stimulated actions and publicity which may not be warranted by the information at hand. It is possible, for example, to achieve 100 percent contamination of some aquaculture stock by a pathogen such as Vibrio parahaemolyticus, which could then attack humans. Many events are possible, but their probability is low if we are aware of the dangers.

The real danger to humans may be exaggerated by newspaper accounts. This may be the case with the anisakiasis "problem" in the United States, which seems to be of vanishingly small significance in a country which eats finfish cooked or otherwise thoroughly processed. Human infection results from eating raw or inadequately processed fish flesh containing the larval worms. "Anisakiasis" as the disease is called, is a problem principally in Japan, where fish are often eaten raw. Control measures, principally in the form of required freezing of herring, have virtually eliminated the problem in northern Europe. Thus far only six cases have been diagnosed in the United States.

The advice given in an editorial in Science (Singer, 1970) about doomsayers might well be heeded. The concluding sentences of the editorial are: "Scientific credibility can easily be lost by exaggerated claims and extravagant statements. We need to provide a voice of reason, not just of alarm. As scientists, we have the responsibility to speak up, but we also must know when to stop talking."

RECOMMENDATIONS

The breadth and depth of subjects included in the program of this regional symposium effectively illustrate the extent of commitment of a number of organizations and a large group of investigators to aquatic animal disease research. Substantial investment and accomplishment in this dynamic area of study is evident. Despite this, there are areas where performance could be improved.

1) Communication should be improved. Symposia such as the present one are important, as are aquatic disease sessions at meetings such as the Wildlife Disease Association and the American Fisheries Society. It seems though that the aquatic animal disease research community is now ready for an Aquatic Animal Disease Society with its own journal. Membership would include all those interested in aquatic animal diseases. At present most aquatic disease specialists are only adjuncts to a number of societies -- Wildlife Disease Association, American Fisheries Society, Society for Invertebrate Pathology, World Mariculture Society -- and many of them are actually excluded from full membership in certain

societies (International Association for Aquatic Animal Medicine). Sections of the proposed new society could be established for each of the major animal groups -- so, for the first time, all those interested in disease problems in any aquatic animal would have an organization of their own and a journal of their own. The first steps to form AADS could be a tangible result of this symposium.

2) Response to critical disease problems should be improved. The need here is for diagnostic services, possibly financed by Sea Grant at first, then eventually as a profit-making venture. Included would be virology, bacteriology, histopathology, mycology, and chemistry. Several Sea Grant-supported groups have offered such services, but progress seems very slow. Regional diagnostic centers, similar to the Southern Cooperative Fish Disease Project funded by eight southern states and based at Auburn University, might be an alternate feasible approach.

3) A general computerized registry and referral center for diseases of aquatic animals should be established. This would be a major and continuing undertaking, and might best be subdivided among several agencies and institutions. It should be designed to process, catalog, store, and retrieve specimens, histological slides, transparencies, photographs, and literature.

4) Development of control measures for diseases of cultured food species should be accelerated. FDA clearance for chemical treatments in aquaculture should also be accelerated. Chemicals at the top of the list include the nitrofurans and chloramphenicol.

5) Finally, a broader information base derived from research is needed. Progress is needed in such areas as epizootiology, histopathology, and environmental stress effects on disease. In this matter, NOAA's Office of Sea Grant should be commended for its enlightened support of aquatic disease studies in various universities around the country. It is interesting that here in the Southeast, possibly because of its greater aquaculture commitment, there seems to be more activity in aquatic disease research than in most other parts of the country. Symposia such as this one are vital ingredients in the progress of this or any comparable area of science.

LITERATURE CITED

Carmody, D., J. Pearce, and W. Yasso. 1973. The distribution of five heavy metals in the sediments of New York Bight. Mar. Poll. Bull. 4:132-5.

Mahoney, J. B., F. H. Midlige, and D. G. Deuel. 1973. A fin rot disease of marine and euryhaline fishes in the New York Bight. Trans. Amer. Fish. Soc. 102:596-605.

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

Shotts, E. B., J. L. Gaines, L. Martin, and A. K. Prestwood. 1972. Aeromonas-induced death among fish and reptiles in eutrophic inland lake. J. Amer. Vet. Med. Assoc. 161:603-7.

Sindermann, C. J. 1970. Diseases of marine animals transmissible to man. Lab. Med. 1:51-4.

Singer, S. F. 1970. Will the world come to a horrible end? Science 170:1637.

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

Southern California Coastal Water Research Project. 1973. The ecology of the Southern California Bight: implications for water quality management. Report no. SCCWRP-TR 104. 531 p.

Young, P. H. 1964. Some effects of sewer effluent on marine life. Calif. Fish and Game 50:33-41.

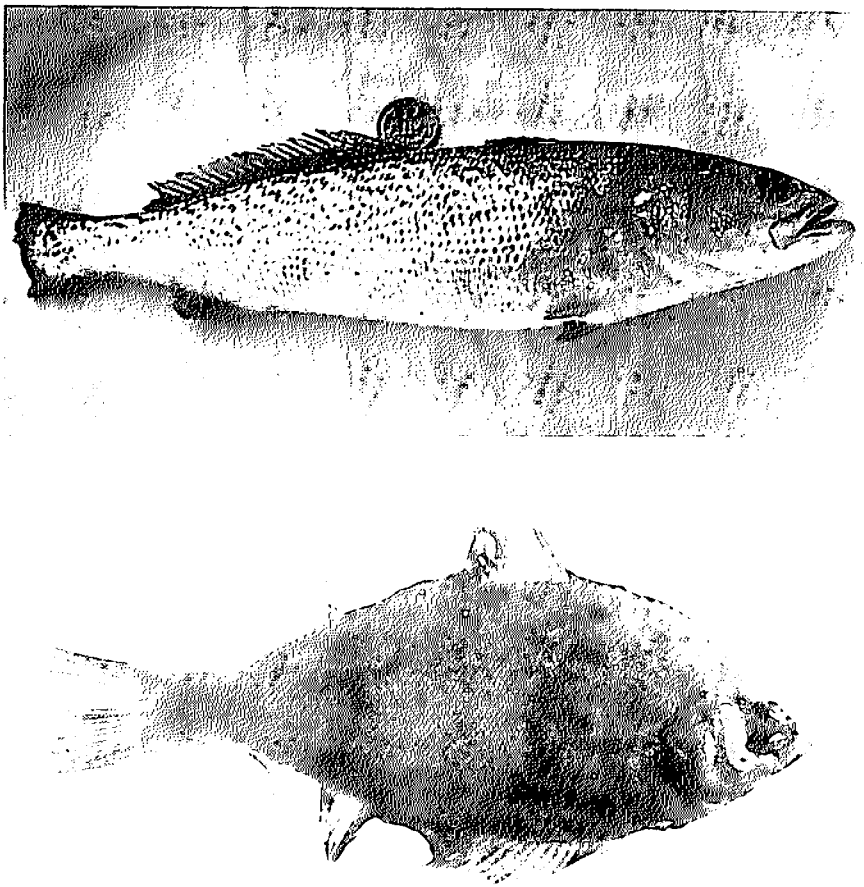

Figure 1. "Fin rot" in sea trout (above) and winter flounder (below).

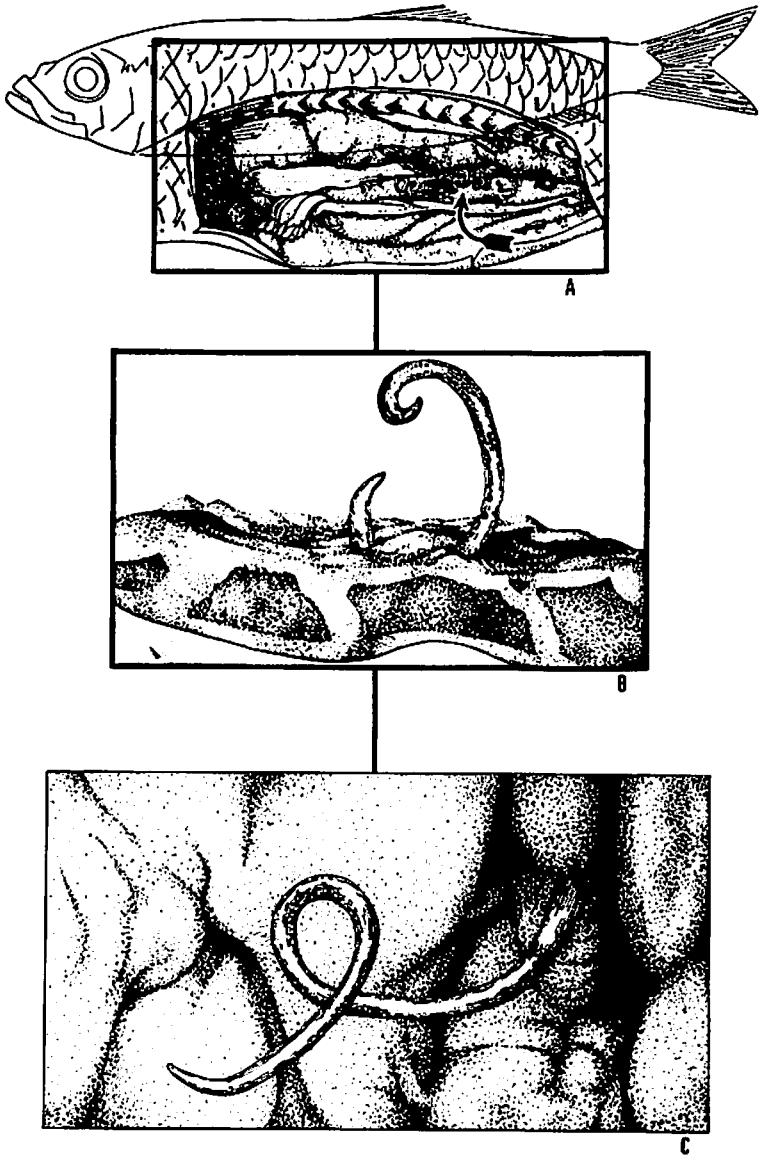

Figure 2. Anisakiasis. Larval worms in viscera of sea herring (top); larval penetration of body muscle of herring (center); and larval invasion of of human intestine (below). (From Sindermann, 1970.)

INTRODUCTORY SPEECH

DISEASES OF AQUATIC ANIMALS AND THE VETERINARIAN

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I propose to speak of the role veterinary medicine appears to be assuming in the conservation and management of aquatic and marine animal resources. The discourse will be viewed from a general standpoint and will be limited in scope and perspective. The mass of information on aquatic and marine biology is extensive and references are increasing concerning the matter of fish production, fish research, fish pathology and the management of fish health in cultural programs or enterprises. The diversity of interests is exemplified by the titles listed in the program of this Symposium.

It would be useful at the outset to recognize that veterinary medicine in the United States and elsewhere enjoys a unique social position. This is so by virtue of the fact that veterinary medicine is simultaneously a health science, an agricultural science, and a general biological and environmental science. This diversity supports the uniqueness of the profession and provides the foundation for future societal service. Modern veterinary medicine has emerged over the last half century through the efforts of many talented individuals and as a result of their dedication has become recognized as the field of study concerned with disease and health of non-human animals. Our horizons are broad but our outreach is diminished by a limited manpower resource, currently at 27,000 graduates in the United States.

The history of the profession of veterinary medicine is replete with major successes and a few failures gained in fulfilling responsibilities to mankind as an agricultural science. As our parental root, the principal professional emphasis until just after the turn of the century was on the enhancement of the well-being of man by assisting in alleviating past and impending food shortages, especially animal protein, and by assisting in the improvement of human malnutrition on a global basis. These contributions have been accomplished through a continuing ability to generate new

information on animal diseases, primarily those of domesticated animals and through the application of information in the evaluation and formulation of new approaches to livestock disease prevention and control.

The extensive and intensive propagation of fish through recently developed aquaculture and marine culture systems has brought a new dimension to veterinary medicine that is exciting, challenging, and attractive to many highly capable young graduates. The interest and activity among a small group of veterinarians during the last decade is similar in retrospect to the establishment of the specialty of Laboratory Animal Medicine which occurred during the mid to late 1950's. (Today LAM is one of the most dynamic of more than 30 specialty fields in the profession). I am of the opinion that graduate veterinarians are not only qualified to address their professional capabilities to controlling infectious and non-infectious diseases of managed or wild aquatic food animals but will increasingly participate in these activities from the private and public practice standpoint.

From what I have learned from my limited review of this subject, the potential for expansion of the aquaculture industry is dependent on the development of acceptable and economical intensive culture systems. These will involve large or small production units, intensive management, dense stocking, stock selection, and stock manipulation. These prerequisites are similar to those required for success in any managed animal population and perhaps foretell the sanitary and disease problems that may be encountered. Epidemiologic studies of managed or manipulated animal populations indicate that disease within a population is frequently a function of the magnitude of stress factors, of the intensity of population numbers, and of the quality of the environment to which the population is exposed.

The fish population in a propogation unit is similar in many respects to an animal herd, and the same principles that apply to herd management apply to fish management. Techniques of application may differ however. I suppose that when solutions are found to current disease problems associated with aquaculture enterprises, herd health management principles will be modified and applied to reduce morbidity and mortality in the culture population. Recently Dr. George W. Klontz estimated that 30 cents of each dollar spent to raise fish goes for some aspect of disease control. This is a sizable dollar loss to a fledgling industry attempting to produce an economically acceptable product. It is apparent that disease prevention methods must be developed that are applicable on a population basis.

Preventive medicine entails development of immunologic procedures for the important viral and bacterial diseases, establishment of diagnostic laboratories with the capability of detecting

identifiable disease carriers, establishment of regulatory measures for the elimination of carrier animals, implementation of effective managerial and nutritional practices, and establishment of a fish health code for restricted transport of diseased or carrier stock across state or regional boundaries.

Recently The Subcommittee on Aquatic Animal Health of the National Research Council Agricultural Board Committee on Animal Health issued a list of recommendations, among which was the call for an increase in the number of qualified individuals to deal with problems of aquatic animal health, for an increase in the research effort on existing and future defined disease problems of aquatic animals, for coordination of federal and state regulations to control the movement and transfer of animals, for the establishment of a national fish and shellfish disease recording and reporting center, and for the development of adequate education and training programs to produce personnel competent to prevent, diagnose, control, and conduct research on diseases of aquatic animals. Recently, animal health research legislation, sponsored by organized veterinary medicine, passed both houses in Congress and included provisions for monetary support of projects involving fish and shellfish disease investigation.

Several months ago, the Council on Public Health and Regulatory Veterinary Medicine of the American Veterinary Medical Association issued a position statement on the role of veterinary medicine in aquaculture. According to the statement, " The profession should be prepared to provide expertise in aquaculture to both individual practitioners and government veterinarians in those specific geographic areas where such expertise, in husbandry, harvesting, food processing and education, is warranted." The Council recommended that the various schools should provide training at the undergraduate or graduate professional level in order for students to gain such expertise.

Only last month, hearings were held in the House of Representatives on proposed legislation to include the inspection of fish and fish produces under a program similar to the voluntary poultry inspection program of several years ago. The AVMA and the NAFV testified in favor of such legislation.

The health science responsibility of veterinary medicine while broad in perspective primarily deals with the establishment of control and research programs dealing with the zoonoses. While there are over 200 zoonotic diseases identified in the animal populations on a worldwide basis, fortunately very few are known to occur within aquatic or marine animal populations. For those zoonotic diseases or infections of animals that are edible, the chance of human infection is directly related to whether the produce is consumed raw or cooked. From this standpoint, shellfish probably

will receive the greatest amount of attention. Proper cooking apparently takes care of those disease agents capable of being transmitted by fish through mechanical or biological means. While cooking provides a safety factor, it does not diminish the importance of the biological association nor does it preclude the development of an intensive program to identify the importance of potential diseases or public health hazards. Apparently, the current literature contains many unsubstantiated speculative references to the role of fish as potential carriers of human disease. The conservative stance assumed two decades ago may be applied to this area, i.e., the potential for human infection from an animal disease agent must be assumed until the agent is defined and its affect on man found to be innocuous or incriminated as a hazard.

Veterinary medicine has been concerned with the identification of animal models for human disease. The importance of these research tools is obvious. A recent article by one of the School's faculty members, Dr. Sheldon Biven, reviews the anatomic and physiologic characteristics of several marine animals that make them of special value as research animals models. This aspect of aquatic and marine biology needs further study and possibly expansion.

The general biological and environmental science role of veterinary medicine is the most recent to receive attention by the profession. It is the least developed area of the profession's social responsibility and projects the outreach to free living or wild animals, these to include fish, shellfish, and invertebrate animal populations. Many persons outside veterinary medicine are working in these fields. Their contributions are and will continue to be important, but obviously so are those of the one profession whose training is in animal pathology, in epidemiology, and in those other biomedical sciences and clinical skills which specifically qualify the veterinarian as the student of animal diseases.

A few years ago, Dr. Calvin W. Schwabe of the University of California stated: "Priorities for veterinary programs must be allotted on the basis of their effects upon the total economy and, in particular, upon preventable losses in protein foods of high quality and in other animal products of great economic value. In this perspective, the value of eliminating or at least of bringing under control the epidemic and even the more insidious and costly endemic diseases of livestock must be measured in terms of their ultimate impact upon the food and health needs of the entire human population of the world. The establishment of an important international center for foot-and-mouth disease research in Brazil under the sponsorship of the Pan American Health Organization is but one indication of how farsighted public health planners have begun to grasp the significance of this relationship to man's health and well-being.

"It is my opinion, however, that most faculties of veterinary medicine in the United States have yet to grasp the significance of this relationship and are for the most part still unprepared to accept national and international responsibilities such as these as being paramount to our profession's continued effective service. Instead, many of our veterinary schools continue to define their role and their goals almost exclusively in terms of the demand imposed by the continuing and important need for veterinarians in private practice."

During the past four years, the attitudes of faculties in veterinary medicine have changed considerably. The flux that is evident in curricular revision and the great activity by curriculum committees in reordering the priorities of veterinary medical education are an indication of what will emerge as the new image of veterinary medicine. Several schools of veterinary medicine have initiated instructional programs in zoologic medicine, pathologic zoology, fish medicine, diseases and pathology of exotic and wild animals, etc., and several have established diagnostic laboratory services that are necessary for effective disease control and prevention in all species of animals.

Members of the faculty of veterinary medicine at Louisiana State University have grasped the significance of the broadening veterinary relationships to man's health and well-being and are committed to expand the perspectives of social responsibilities in all aspects of veterinary medical services.

FACTORS INFLUENCING THE BACTERIAL DISEASES OF POIKILOTHERMIC AQUATIC ANIMALS[1]

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INTRODUCTION

The nature of diseases of poikilothermic aquatic animals and the problems associated with them are well documented (Reichenbach-Klinke and Elkan, 1965; Sindermann, 1970; Inman and Hambric, 1970; Mawdesley-Thomas, 1972; Van Duijn, 1973). This mass of literature is a collection of facts on infectious diseases caused by parasites, viruses, bacteria, fungi, and protozoa as well as non-infectious diseases including tumors and diseases resulting from nutritional deficiencies. However, living creatures in nature do not exist in isolation but are exposed to numerous external physical, chemical, and biological factors as well as the effects of internal factors including the normal physiological and ageing processes. These factors not only affect the host and the potential pathogens but the interaction between the host and the pathogen as well. Thus disease occurs only under specific favorable ecological conditions. The diagnosis of the cause of death in an aquatic animal is not always obvious. Even in the case of obligate pathogenic bacteria of fish such as Aeromonas salmonicida (the causative agent of fur-

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unculosis) and Hemophilus piscium (the causative agent of ulcer disease), immunologic and genetic resistance plays an important role in the outbreak of epizootics (Snieszko, 1964).

The long term studies necessary to solve some of these problems and at the same time avoid needless duplication have been acknowledged. Toward this goal a recent survey designed to identify the major problems of fish health was conducted in the United States (Hester, 1973). Each state, each regional federal laboratory, and several commercial fish producers provided data for the survey. Current problems in fish health were identified according to the frequency and severity of the problem. Those problems which were reported with the greatest frequency and their final cumulative point rankings are presented in Table 1.

With perhaps the exception of bacterial gill disease, which was the highest priority problem, the variations in total point value and subsequent rankings are not by themselves significant. It is evident however that more than 50 percent of the major infectious diseases of fish in the United States are the apparent result of bacterial infection. In addition to these results, myxobacterial disease of cold-water fishes and bacterial redmouth disease, both reported in low frequency, usually produced serious kills and extremely heavy losses of infected fish.

The etiologies of other diseases of poikilothermic animals have not been unequivocally determined. It has been suggested that infectious dropsy in carp is actually a disease complex associated with Rhabdovirus carpio (Fijan, 1972) and that the clinical symptoms of classic red-leg disease of frogs thought to be caused by Aeromonas hydrophila may actually represent a generalized response to bacterial infection (Gibbs, 1973, and Amborski and Glorioso, 1973). Chitinoclastic bacteria have been isolated from crustacea demonstrating shell disease, but these isolates have not reproduced the disease experimentally. The problems associated with the etiology of diseases of aquatic animals will continue to be frustrating, although the role of bacteria as major pathogens is indicated.

The mass of data collected on fish diseases certainly reflects the economic importance of the animal. Table 2 presents the host distribution of known bacterial pathogens of aquatic animals, and the number of potential fish pathogens is evident. As more data is accumulated with other aquatic animals, this list will expand.

This list shows that 28 genera of bacteria have been shown to be active in poikilothermic animals. Seventeen of these genera are apparently pathogenic for one host, and of these, 12 genera are specific for fish. Among the factors that play important roles in the distribution and effect of these pathogens are:

1) Tissue affinity as in the case of gill disease caused by myxobacteria and the requirement for chitin as in the case of shell disease of shrimp, crayfish, and lobster (Cook and Lofton, 1973).

2) Host-species specificity as in the case of Pasteurella pfaffi infection of the white perch Roccus americanus (Snieszko and Bullock, 1964 and Van Duijn, 1973).

3) The requirement for a specific developmental stage of the host as in the case of Proteus mirabilis infection of the bullfrog tadpole (Glorioso et al., 1974) and Vibrio sp. infection of the larvae and juvenile forms of bivalve species (Guillard, 1959; Tubiashi et al., 1965).

4) Environmental limitation as in the case of Vibrio anguillarium, a truly marine pathogen limited to salinities above nine ppt.

5) The apparent complete resistance to most potential bacterial pathogens as in the case of shrimp, crayfish, and lobster even though their activities would certainly expose these animals to many of the listed pathogens.

In contrast to these observations, species of Aeromonas, Mycobacterium, and Pseudomonas are apparently common pathogens in many aquatic animals. Furthermore, in the host systems most extensively studied, i.e. fishes and frogs, species of Corynebacterium and Flavobacterium have been identified as important pathogens. These five genera represent a group of organisms generally referred to as opportunistic pathogens.

Any attempt to understand and control the bacterial disease processes in poikilothermic aquatic animals must take into consideration the fact that most healthy animals in nature and in artificial communities harbor many potentially pathogenic organisms. Such organisms are especially prevalent in the alimentary tract, and disease results only under as yet undefined conditions. Numerous factors are important in disease production by opportunistic pathogens and these include:

1) Distribution of pathogens

2) Immune response of the host

3) Indigenous flora of the susceptible host

4) Toxin production by the pathogen

5) Stress.

DISTRIBUTION OF PATHOGENS

It is evident that all natural waters contain living bacteria. The sources of bacteria found in the water include:

1) Organisms washed from the atmosphere and surfaces of vegetation by precipitation.

2) Organisms washed from the soil by surface water runoff.

3) Organisms excreted in the feces of both warm and cold-blooded animals.

With organisms coming from such diverse sources, it would be expected that wide variations in the bacterial content of water exist. The number and type of bacteria vary according to the source, the organic matter content, and the mineral content of the water. Fairly pure waters support few total numbers of microorganisms but have a relatively large number of different species. Thus although many organisms found in the aquatic environment can be considered as transients, water, even distilled water, has a substantial growth potential. Among the bacteria which can reproduce in water of unquestioned potable quality are: Pseudomonas, Xanthomonas, Achromobacter, Escherichia, Aerobacter, Streptococcus, Desulfovibrio, and Aerothrix (Chambers and Clarke, 1966). As more contaminants enter the water, the total number of microorganisms increases, while the number of species decreases. Numerous workers have confirmed the fact that a variety of Pseudomonas species can utilize several unusual carbon sources. Among these sources are aliphatic and aromatic hydrocarbons, alcohols, phenols, plasticisers used in the plastics industries, and chlorinated hydrocarbon insecticides (Holden, 1970). Aeromonas liquefaciens is found in large numbers in water bearing a heavy organic burden. Furthermore Aeromonas sp. have been found in surface water in larger numbers than E. coli or Enterobacter sp., and the Aeromonas sp. grow in the sludge in sink traps and in sewage along with Pseudomonas sp. and Myxobacteria sp., also important as pathogens of aquatic animals. Recovery of almost pure cultures of Aeromonas sp. from the effluent of sewage-treatment and citrus-processing plants has been reported (Shotts et al., 1972).

These observations have thus led to the acknowledgment that many of the important bacterial pathogens of aquatic animals exist as free-living forms. In the case of the Pseudomonads specifically, it is becoming increasingly difficult to make a clear distinction between animal pathogens, plant pathogens, and the free-living forms. Thus Pseudomonas aeruginosa is a major animal pathogen, but apparently identical strains cause disease in sugarcane, tobacco, and lettuce. Pseudomonas cepacia, first described as a cause of sour skin in onion bulbs, is widespread in the soil and

drinking water and has caused several outbreaks of infection in man (Parker, 1971).

One of the more recent studies on the distribution of pathogenic bacteria in two lakes further supports some of the previous observations (Collins, 1970). Those bacteria which are recognized as opportunistic pathogens of various species of fish were found in the epilimnion. The specific organisms isolated included Pseudomonas sp., Aeromonas sp., Myxobacteria sp., and Flavobacterium sp., while Corynebacterium sp. were often found in the thermocline. Bacteriological examination of apparently healthy animals as well as animals demonstrating clinical symptoms of disease showed that the above group of pathogens could be easily isolated from the surface and tissues and organs of both groups of fish. Bacteriological analysis of healthy fish identified Pseudomonas sp. and Aeromonas sp. as the most common bacterial inhabitants on the surface of fish. Myxobacteria sp. were the most numberous organisms isolated from the gills. In the internal organs, the liver contained a number of gram positive organisms, and the most predominant isolate was an unidentified Corynebacterium sp. Few, if any, bacteria could be isolated from the kidney or heart blood, but Aeromonas sp. and Pseudomonas sp. could be isolated from the peritoneal cavity. In animals showing clinical symptoms of disease, Aeromonas sp., Pseudomonas sp. and Myxobacteria species were routinely isolated from the external slime and from the gills. Pseudomonas sp. were also found in the peritoneal cavity.

IMMUNE RESPONSE OF THE HOST

During the past 15 years, numerous studies have supported the concept that immune responses within vertebrates have a basic common mechanism. All vertebrates including fishes, amphibians, and reptiles possess lymphocytes, synthesize immunoglobulins of a fundamentally identical structure, and reject allografts (Du Pasquier, 1973). Acquired resistance by means of immunoglobulins has not been demonstrated in crustacea or other invertebrates. However, induced or enhanced bacteriocidin formation has been described in lobsters after injection of an antigen (Unestam, 1973). In another report the level of resistance in Australian crayfish to Pseudomonas infection was increased by vaccination (McKay and Jenkin, 1969), and resistance to the crayfish plaque fungus was increased by injection of sublethal doses of the fungal spores (Unestam and Weiss, 1970). Thus mass mortality of poikilothermic aquatic animals may not simply represent the lack of immunological responses to potential microbial pathogens. Furthermore as we expand our knowledge about the immune response in fishes, amphibia, and crustacea, it may be possible to stimulate these activities by vaccination. Klontz and Anderson (1970) have recently reviewed attempts to immunize orally trout and salmon against two infectious

bacterial diseases, red mouth and furunculosis. Although protection was achieved by the oral administration of phenol-killed or chloroform-killed bacteria, the results were equivocal and additional studies on this method of control are obviously necessary.

INDIGENOUS FLORA OF THE ANIMAL

Of the numerous bacteriological analyses on diseased aquatic animals, the major emphasis has been placed on the major pathogens which could be isolated from the individual animals. With the demonstration that the opportunistic pathogens could be isolated from the intestinal tract of healthy animals, it has been suggested that such disease processes could be initiated in the intestinal tract. The growth of these organisms and the elaboration of toxins that damage host tissues could give rise to characteristic symptoms of disease, and bacteria might occur in the tissues and blood only during the late or terminal stages of the disease. These mechanisms operate in several human diseases but indigenous microorganisms are known to interfere with such infections (Savage, 1972). In the upper respiratory tract, infections by staphylococci, streptococci, and gram negative bacteria have been shown to be limited by the indigenous flora. In the gastrointestinal tract similar effects inhibit infection by V. cholerae, Shigella sp. and Salmonella sp.

The control exerted by the indigenous flora can be artificially demonstrated by antibiotic treatment. In our laboratories, within 48 hours after tetracycline treatment, normal frogs demonstrated a fatal septicemia with a tetracycline resistant Flavobacterium sp. (Glorioso et al., unpublished results). Inasmuch as this same organism could be isolated from the feces of the animals before antibiotic treatment, the results would suggest a role for the tetracycline sensitive organisms in controlling the activities of the Flavobacterium sp. As one might expect, the bacterial isolates from normal and diseased frogs included a non-pathogenic group as shown in Table 3 as well as a pathogenic group as shown in Table 4. These studies have not been extensive enough to determine the indigenous microbiota of frogs, but the results do indicate the complexity of possible interactions.

TOXINS PRODUCTION BY THE PATHOGEN

It has generally been accepted that pathology due to gram negative bacteria is totally attributable to endotoxins. However, some gram negative human pathogens produce protein toxins that are not related to the cell wall. Examples include toxins of

Shigella dysenteriae, Bordetella pertussis, Yersinia pestis, Vibrio cholerae, and Pseudomonas pseudomallei (Liu et al., 1973). In addition to these observations, exotoxins have been isolated and purified from Pseudomonas aeruginosa (Liu, 1973), and in our laboratories nutrient broth cultures from which the bacterial cells were removed by a combination of centrifugation and filtration were shown to produce products lethal for frogs, fish, and crayfish as shown in Table 5.

However, in assessing the production of toxic factors by bacterial cells, one must consider the toxic effects of nitrogenous metabolites, especially ammonia. Ammonia has been demonstrated to be a potent neurotoxin for poikilothermic aquatic animals (Amlacher, 1970). Such problems can be overcome by column chromatography as shown in Figure 1, and complete removal of ammonia can be achieved.

The factors required for toxin production have not as yet been determined and are of obvious importance in determining the role of such toxins in various disease processes.

STRESS

Stress, which either lowers the resistance of the host or enhances the pathogenicity pathogen, is important in the disease process. Under the restricted conditions of many artificial culture systems, problems of nutrition, anoxia, hypoxia, fright, exertion, crowding, handling, temperature change, and injury are among the factors which place added burden on cultured animals. In response to these effects numerous morphological, biochemical, and physiological changes are initiated in the animal. The most typical component of a stress reaction is activation of the adrenal cortex with an increased level of corticosteroids in the plasma and adrenal glands (Selye, 1973). These effects can be shown in fishes and amphibia (Wedemeyer, 1970), and the measured responses include the following:

1) Depletion of ascorbic acid

2) Inhibition of release of antibody

3) Increased serum glucose

4) Disturbances of osmotic or ionic regulation

5) Decrease in circulating lymphocytes

Unfortunately the biochemical and physiological effects of all the stress-mediated changes are not fully understood and the exact role of the known effects of stress on the production of disease are unknown. These problems are exemplified by the attempts of Bullock (1972) to identify the environmental conditions necessary for the induction of bacterial gill disease in salmonids. Exposure of fish to 0.5, 1.0, or 2.5 ppm ammonia or to low oxygen levels (4 to 5 ppm) or to both ammonia and low oxygen did not induce gill disease, even when myxobacterial cultures were added to the aquarium water in the form of pure cultures or dead diseased fingerlings. However, when live diseased fingerlings were added to the stressed fish, gill disease appeared in three to five days but did not appear in control fish either with or without the addition of diseased fingerlings. The nature of the contribution of the live diseased fingerlings is not known, but these results suggest that stress is not the only answer to disease production by opportunistic pathogens.

Obviously the factors responsible for disease production by opportunistic bacteria are complex and probably not mutually exclusive. However as we identify more of the significant factors, new levels of understanding can be achieved and alternate means of disease control might be developed.

LITERATURE CITED

Amborski, R. L., and J. C. Glorioso. 1973. The frog revisited. Science, 181:495.

Amlacher, E. 1970. Textbook of fish diseases. TFH Publications. Jersey City, N. J. pp. 244-63.

Borg, A. F. 1960. Studies on myxobacteria associated with diseases in salmonid fishes. J. Wildlife Disease, 8:1-85.

Bullock, G. L. 1972. Studies on selected myxobacteria pathogenic for fishes and on bacterial gill disease in hatchery-reared salmonids. Technical Papers of the Bureau of Sport Fisheries and Wildlife, No. 60. pp. 1-30.

Chambers, C. W., and N. A. Clarke. 1966. Control of bacteria in non-domestic water supplies. Advances in Applied Microbiology 8:105-43.

Collins, V. A. 1970. Recent studies of bacterial pathogens of fresh water fish. Water Treatment and Examination, 19:3-31.

Cook, D. W., and S. R. Lofton. 1973. Chitinoclastic bacteria associated with shell disease in penaeus shrimp and the blue crab. Journal Wildlife Diseases, 9:154-9.

Du Pasquier, L. 1973. Ontogeny of the immune response in cold-blooded vertebrates. Current Topics in Microbiology and Immunology, 61:37-88.

Fijan, N. A. 1972. Infectious dropsy in carp - a disease complex. In Diseases of Fish (L.E. Mawdesley-Thomas, ed.). Academic Press. pp. 39-51.

Gibbs, E. L. 1973. Healthier frogs. Science, 181:1201.

Glorioso, J. C., R. L. Amborski, G. F. Amborski, and D. D. Culley. 1974. Microbiological studies on septicemic bullfrogs (Rana catesbeiana). American Journal of Veterinary Research (in press).

Guillard, R. R. L. 1959. Further evidence of the destruction of bivalve larvae by bacteria. Biological Bulletin, 117:258-66.

Hester, E. F. 1973. Fish health: A nationwide survey of problems and needs. Progressive Fish Culturist, 35:11-8.

Holden, W. S. (ed). 1970. Water treatment and examination. Williams and Wilkins Co., Baltimore. pp. 223-30.

Inman, C. R., and R. N. Hambric. 1970. Diseases and parasites of warm-water fishes. Texas Parks and Wildlife Department Technical Series, No. 2. 55 pp.

Klontz, G. W., and D. P. Anderson. 1970. Oral immunization of salmonids: A review. In A symposium on diseases of fishes and shell fishes. S.F. Snieszko (ed.). American Fisheries Society, Washington, D. C.

Liu, P. V. 1973. Exotoxins of Pseudomonas aeroginosa. I. Factors that influence the production of exotoxin A. Journal of Infectious Diseases, 128:506-13.

Liu, P. V., S. Yoshii, and H. Hsieh. 1973. Exotoxins of Pseudomonas aeruginosa. II. Concentration, purification, and characterization of exotoxin A. Journal of Infectious Diseases, 128:514-9.

Mawdesley-Thomas, L. E. (ed.). 1972. Diseases of fish. Academic Press. New York. 380 pp.

McKay, D., and C. R. Jenkin. 1969. Immunity in invertebrates. II. Adaptive immunity in the crayfish (Parachaeraps bicarinatus). Immunology, 17:127-137.

Parker, M. T. 1971. Causes and prevention of sepsis due to gram-negative bacteria. Proceedings Royal Society of Medicine, 64:979-80.

Reichenbach-Klinke, H., and E. Elkan. 1965. The principal diseases of lower vertebrates. Academic Press, New York. 600 pp.

Savage, D. C. 1972. Survival on mucosal epithelia, epithelial penetration and growth in tissues of pathogenic bacteria. Symposium of the Society for General Microbiology, 22:25-58.

Selye, H. 1973. The evolution of the stress concept. American Scientist, 61:690-9.

Shotts, E. B., J. L. Gawes,Jr., L. Martin, and A. K. Prestwood. 1972. Aeromonas-induced deaths among fish and reptiles in an eutrophic inland lake. Journal American Veterinary Medical Association, 161:603-7.

Sindermann, C. J. 1970. Principal diseases of marine fish and shellfish. Academic Press, New York. 367 pp.

Snieszko, S. F. 1964. Remarks on some facets of epizootiology of bacterial fish diseases. Developments in industrial microbiology, No. 5 (C. F. Koda, ed.). Garamond/Pridemark Press. Baltimore Maryland. pp. 97-100.

Snieszko, S. F. (ed.). 1970. A symposium on diseases of fishes and shellfishes. Special publication No. 5, American Fisheries Society, Washington, D. C. 526 pp.

Snieszko, S. F., and G. L. Bullock. 1964. A massive kill of white perch (Roccus americanus) involving a Pasteurella-like bacterium. Bact. Proc., G 154.

Tubiashi, H. S., P. E. Chanley, and E. Leifson. 1965. Bacillary necrosis, A disease of larval and juvenile bivalve mollusks. I. Etiology and epizootiology. J. Bacteriol, 90:1036-44.

Unestam, T. 1973. Fungal diseases of crustacea. Review of Medical and Veterinary Mycology, 8:1-20.

Unestam, T., and D. W. Weiss. 1970. The host-parasite relationship between freshwater crayfishand the crayfish disease fungus Aphanomyces astaci: Responses to infection by a susceptible and a resistant species. Journal of General Microbiology, 60:77-90.

Van Duijn, C. 1973. Diseases of fishes. Charles C. Thomas. Springfield, Illinois. 372 pp.

Wedemeyer, G. 1970. The role of stress in the disease resistance of fishes. In A symposium on diseases of fishes and shellfishes (S. F. Snieszko, ed.). American Fisheries Society, Washington, D. C. pp. 30-5.

Table 1. Priority ranking of fish diseases

Disease	Cumulative Point Value
Bacterial gill disease	100
Aeromonad and Pseudomonad infections (excluding *Aeromonas salmonicida*)	77
Ichthyophthirius	73
Furunculosis	66
Ectoparasites (excluding Ichthyophthirius)	64
Infectious pancreatic necrosis virus	63
Corynebacterial disease	56

Table 2. Host distribution of known bacterial pathogens to aquatic animals

PATHOGEN	HOST									PATHOGEN	HOST								
	Fish	Turtle	Snake	Alligator	Frog	Oyster	Shrimp	Crayfish	Oyster		Fish	Turtle	Snake	Alligator	Frog	Oyster	Shrimp	Crayfish	Lobster
Acromobacter sp.						*				Mycobacterium thamnopheos			*						
Aeromonas hydrophila		*	*	*	*					Nocardia asteroides	*								
Aeromonas liquefaciens	*				*	*				Pasteurella sp.	*								
Aeromonas salmonicida	*									Proteus mirabilis					*				
Aeromonas sp.						*				P. rettgeri					*				
Arizona sp.		*								P. vulgaris	*			*	*				
Chitinoclastic bacteria							*	*	*	Pseudomonas aeruginosa					*				
Chondrococcus columnaris	*									P. fluorescens	*		*						
Citrobacter freundii		*			*					P. putida					*				
Clostridium sp.	*									P. reptilivora				*					
Corynebacterium sp.	*				*					Pseudomonas sp.			*	*	*				
Cytophaga psychrophila	*									Salmonella marina				*					
Edwardsiella tarda	*									Salmonella sp.	*	*		*					
Erysipelothrix sp.	*									Serratia piscatorum	*					*			
Flavobacterium sp.	*				*					Staphylococcus epidermidis					*				
Gaffkya homari									*	spirochete sp.	*								
Haemophilus piscium	*									Streptococcus sp.	*								
Leptospira pomona		*	*							Streptomyces sp.	*								
Micrococcus sp.		*	*	*						Vibrio anguillarium	*					*			
Mima (Acinetobacter) sp.					*					V. parahaemolyticus	*								
Mycobacterium chelonei		*								V. shigelloides	*	*		*	*				
Mycobacterium platypoecilus	*									Vibrio sp.						*			
Mycobacterium ranae				*															

Table 3. Pathogenic bacteria isolated from diseased bullfrogs, Rana catesbeiana

Aeromonas hydrophila
Aeromonas shigelloides
Citrobacter freundii
Flavobacterium sp.
Mima polymorpha
Proteus mirabilis
Proteus morganii
Proteus rettgeri
Proteus vulgaris
Pseudomonas aeruginosa
Pseudomonas fluorescens
Pseudomonas putida
Pseudomonas sp.

Table 4. Nonpathogenic bacteria isolated from diseased bullfrogs, Rana catesbeiana.

Achromobacter sp.
Alkaligenes faecalis
Bacillus cereus
Bacillus megaterium
Bacillus sphaericus
Corynebacterium sp.
Enterobacter sp.
Escherichia coli
Gaffkya sp.
Klebsiella sp.
Micrococcus sp.
Pseudomonas alcaligenes
Sarcina lutea
Serratia sp.
Shigella sp.
Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus sp.
Vibrio sp.

Table 5. Lethal effects of bacterial products on crayfish, fish, frogs, and tadpoles

Preparation	No. Deaths out of 5 Animals			
	Crayfish	Fish	Frogs	Tadpoles
Millipore Filtered Broth Culture				
Pseudomonas aeruginosa	4	3	5	5
Aeromonas liquefaciens	4	3	5	5
Citrobacter freundii	4	5	5	5
Flavobacterium sp.	5	5	5	5
Escherichia coli	0	0	0	0
CM Chromatography Fractions 4 & 5				
Flavobacterium sp. (900 mgm/L ammonia)	3	4	4	5
Sephadex Chromatography Fractions 6 & 7				
Flavobacterium sp. (20 mgm/L ammonia)	3	4	5	5
CM Chromatography Fractions 4 & 5				
Flavobacterium sp. (no ammonia)	3	3	5	5

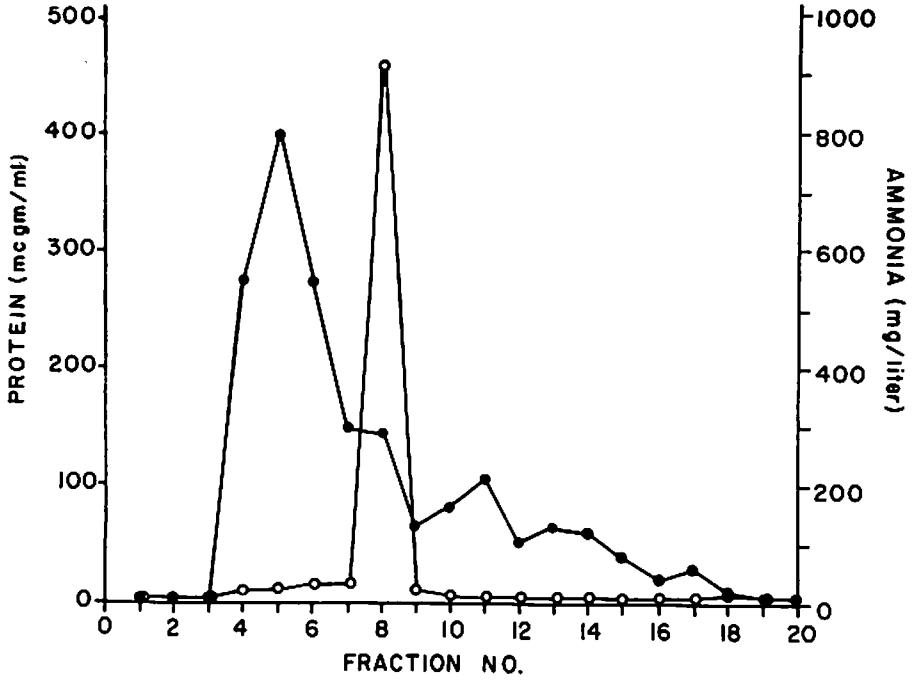

Figure 1. Elution patterns from Sephadex G-15 column of toxic bacterial products. Closed circles indicate micrograms protein; open circles indicate milligrams ammonia.

PSYCHROPHILES

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INTRODUCTION

At any temperature at which water is available in a liquid state, there is some microorganism which is able to grow. In the hot sulfur springs of Yellowstone the superheated water is at about 95 C. and it supports microbial growth. Hot springs in New Zealand and Iceland have temperatures of about 100 C, and they also support microbial growth (Brock and Garland, 1970). At the lower end of the temperature scale there are many microorganisms which are capable of sustaining growth at 0 C. Indeed, many of these will grow at lower temperatures if freezing of the menstruum is prevented (Stokes, 1968). Those organisms which grow at the upper temperature range are called thermophiles while those growing in the low temperature range are called psychrophiles. Those bacteria which grow in the intermediate range of temperature are called mesophiles.

DISCUSSION

Definition of a Psychrophile

There has been considerable controversy over a definition of a psychrophile. Food microbiologists often like to consider anything that grows in the refrigerator and spoils food as a psychrophile. Those microbiologists who are concerned with the mechanisms and biochemical aspects of cell growth do not accept that definition because several bacteria which are normally considered to be mesophilic will grow, albeit poorly, at refrigerator temperatures of

about 4-7 C. A definition which has become widely accepted is that of Stokes (1963) who defined psychrophiles as microorganisms which grow rapidly enough at 0 C to become macroscopically visible as a colony in about one week. He further subdivided them into obligate psychrophiles, with an optimum growth temperature of below 20 C, and facultative psychrophiles, with an optimum of 20 C or above. It was thought for many years that microorganisms fitting the definition of obligate psychrophiles were very rare or did not exist. However, recent observations of bacteria and yeast collected from the oceans and from soil and water in the colder regions of the world have shown that microorganisms with very low temperature optima exist.

Growth Temperature Range of Psychrophiles

In Figure 1, the relationship of the growth rates of an average psychrophile, mesophile, and thermophile are shown as a function of temperature. Although there are overlapping regions it is relatively easy to categorize any isolate into its proper temperature type. Notice that 0 C is not usually the minimum temperature for growth of a psychrophile. Figure 2 shows the growth of psychrophilic Bacillus in an incubator in which the temperature slowly dropped from -5 to -7 C (Larkin and Stokes, 1968). Growth at this temperature is slow but may result in a large cell crop. Some psychrophiles grow at even lower temperatures, with the range down to -10 C being reported on several occasions (Michener and Elliott, 1964; Larkin and Stokes, 1968). Growth below -10 C and down to -34 C has been reported with decreasing frequency, but not confirmed, and seem doubtful in light of theoretical considerations which place the minimum at about -12 C.

At temperatures above freezing, psychrophiles may grow extensively and rapidly as shown in Figure 3 (Larkin and Stokes, 1966). As the temperature is increased up to 20 C, the growth rate increases to a generation time of 2.5 hr. At 25 C growth was slower, and at 30 C there was no growth.

Ecology of Psychrophiles

It was once thought that psychrophiles were generally restricted to the Gram negative bacterial genera Achromobacter, Flavobacterium and Pseudomonas, but increased interest in psychrophiles and their activities has led to the observation that they exist in at least 23 genera, and undoubtedly as the search for psychrophiles continues others will be found. Table 1 lists the bacterial genera which are known to contain psychrophiles. They may be Gram positive or negative rods, cocci, or vibrios. Moreover, several genera listed also contain known pathogens of humans or other animals. Some of these

psychrophiles could be considered to be cold temperature counterparts of known pathogens of man or other warm-blooded animals.

Bacteria are not the only psychrophilic microorganisms. Table 2 lists the major genera of fungi which contain psychrophiles. As with the bacteria, many of these genera are familiar to persons concerned with diseases of animals, as well as to microbiologists. In addition, bacterial viruses have been reported which attack only psychrophiles, or if they cross the temperature line and infect both psychrophiles and mesophiles, they infect mesophiles only at low temperatures (Olsen, 1967; Whitman and Marshall, 1971; Delisle and Levin, 1969).

That psychrophilic bacteria are abundant in fresh water habitats is demonstrated in Table 3. In all samples tested from Washington State, psychrophiles comprised a significant portion of the bacterial population, and in three of four lake water samples they were more numerous than were mesophiles. In the water samples collected in the winter from Louisiana lakes they were abundant and formed a significant part of the population, although mesophiles were more abundant. Psychrophiles were insignificant in other Louisiana habitats. Comparative data from salt and brackish water habitats are sparse, but in view of the fact that the average temperature of the ocean is about 4 C it seems likely that psychrophiles form a significant portion of the microbial population of the oceans.

Psychrophiles as Pathogens

The above data strongly suggest that psychrophilic microorganisms may be involved in pathogenesis of aquatic animals, either as primary pathogens or as secondary invaders. In the latter role there has been almost no research reported, but as primary pathogens there are at least four disease syndromes associated with psychrophiles. These include bacterial gill disease, septicemic diseases of some fish, bacterial cold water disease, and gaffkemia of lobsters.

Bacterial cold water disease affects young salmon during times when the water temperature is low. It is widespread in hatcheries of the Pacific Northwest where it affects yolk sac fry and fingerlings. The optimum temperature for this disease is between 4.4 C and 10 C and raising the temperature to 12.8 C generally results in a decline of the disease (Rucker et al., 1954). The causative organism is a flexi-bacterium, Cytophaga psychrophila.

Hemorrhagic septicemias of fish are apparently caused by several bacteria or viruses, including the psychrophile, Pseudomonas fluorescens (Otte, 1963).

Gaffkemia is a disease which affects both the American and European lobster, causing a fatal septicemia. At temperatures of

3 to 20 C the lobsters may be experimentally infected and their defensive mechanisms are unable to control the infection, but with infection at 1 C the disease may be suppressed. When the temperature is raised to 3 C or above, the disease will ensue in the infected lobster (Stewart and Rabin, 1970).

Bacterial gill disease affects salmonid fish of all ages. In some hatcheries in Washington it occurs in the spring when water temperatures range from 1.7 to 4.4 C. In hatcheries in other areas the disease occurs at higher temperatures, which indicates that more than one infectious agent may be involved. The etiological agent has not been identified with certainty but myxobacteria are suspected (Wood, 1968). The low temperature at which the disease occurs points to a psychrophilic origin.

There are several other diseases of fish in which the causative agent has not been demonstrated. Some of these are undoubtedly caused by psychrophiles, or have psychrophiles as secondary invaders.

As the world need for protein increases we are constantly aware that the greatest available future source is the marine environment. Many attempts have been made to grow marine fish on a commercial scale, and these have met with varying degrees of success. One problem has been the slow rate of growth of the fish. To increase the growth rate, and thus to increase the commercial feasibility of the program, the temperature of the water is often increased. This, however, may make the fish more susceptible to diseases by psychrophiles because the increased temperature allows the bacteria to grow much more rapidly and perhaps overcome the defenses of the fish. Moreover, the increased stress of crowding upon the fish may enhance their susceptibility to microbial invasion. Psychrophilic microorganisms which now are considered inocuous may become primary or secondary pathogens of the fish under stress.

The problem of psychrophiles may be equally severe in an area which has received little attention from American biologists, that is, marine fish which come from very cold regions of the ocean. As we turn our attention towards harvesting or commercial production of fish from Antarctica we find that many of the fish themselves could be considered psychrophilic. For example, three species of Trematomus live in water that is about -1.9 C. They are not known to occur in water with a temperature above 2 C, and their upper lethal temperature limit is about 6 C. At 10 C they die within 2-1/2 hours (Somero and DeVries, 1967). It seems obvious that any infectious diseases these animals may have are caused by psychrophiles.

Techniques for Handling Psychrophiles

It is probable that psychrophilic microorganisms will take on increased importance as disease agents in aquatic animals. Because

of this, it is important to review some of the problems and techniques associated with working with psychrophiles. In the past, many psychrophiles in the environment were missed because incubation temperatures of 30-37 C were routinely used for isolation, although much of the source materials, particularly those of marine origin, were obtained from environments close to 5 C. This was undoubtedly a carry over from work on mesophiles and on human pathogens. The realization that bacteria which grow well at low temperatures are rapidly damaged by what are considered moderate temperatures has led to some specialized procedures for their examination. Many of them are killed rapidly if mixed with molten agar, and this necessitates pouring of agar plates a day or two in advance, incubating them to dry the surface and then chilling them before addition of the inoculum. Pipettes and dilution blanks should be refrigerated until used. Anything which will contact the organisms should be cold. Incubation temperature is extremely important, and for initial isolation incubation should be in a refrigerator at a temperature from 0-4 C to prevent growth of mesophiles.

After isolation the organism may be grown at its optimum temperature or perhaps even at room temperature. However, some psychrophiles, particularly those from very cold regions of the oceans, have such a low maximum growth temperature that all manipulations must be carried out in a cold room.

For suspected viral diseases, cell cultures such as the fat head minnow system can be prepared at 10 C, transferred to a low temperature incubator and inoculated with the suspected material. Psychrophilic viruses should produce visible signs within a few weeks. Thus, other than the requirement for low temperatures throughout their manipulation, psychrophiles are handled in the same manner as are the other temperature groups of microorganisms.

SUMMARY

In the above discussion I have attempted to make several points about psychrophilic microorganisms, and these are summarized below:

1) Psychrophilic microorganisms grow at low temperatures and include almost all types of bacteria and fungi. Moreover, psychrophilic viruses which attack bacteria do exist. Psychrophilic viruses of plants and animals are an unexplored and probably profitable field of study.

2) Psychrophilic microorganisms appear in great abundance in the colder regions of the world, and even in the more temperate climate of Baton Rouge, La., they are abundant in lakes during the winter months.

3) Psychrophilic bacteria are responsible for several diseases

of aquatic fishes and even lobsters. As biologists and microbiologists become trained to search for psychrophiles, it will undoubtedly be found that they are important as primary or secondary pathogens of many animals. This should be especially true of fish under stress in commercial production facilities and those which grow only in cold waters.

4) The techniques for isolating and handling psychrophiles are the same as for any other microorganism except for the necessity of maintaining the sample, media, and utensils in a cold condition during their use.

LITERATURE CITED

Brock, T. D., and G. K. Garland. 1970. Limits of microbial existence: temperature and pH. Science 169:1316-8.

Delisle, A. L., and R. E. Levin. 1969. Bacteriophages of psychrophilic pseudomonads. I. Host range of phage pools active against fish spoilage and fish-pathogenic pseudonomads. Antonie van Leeuwenhoek. 35:307-17.

Larkin, J. M. 1970. Seasonal incidence of bacterial temperature types in Louisiana soil and water. Appl. Microbiol. 20:286-8.

Larkin, J. M., and J. L. Stokes. 1966. Isolation of psychrophilic species of Bacillus. J. Bacteriol. 91:1667-71.

Larkin, J. M., and J. L. Stokes. 1968. Growth of psychrophilic microorganisms at subzero temperatures. Can. J. Microbiol. 14:97-101.

Michener, H. D., and R. P. Elliott. 1964. Minimum growth temperatures for food-poisoning, fecal-indicator, and psychrophilic microorganisms. Adv. Food Res. 13:349-96.

Olsen, R. H. 1967. Isolation and growth of psychrophilic bacteriophage. Appl. Microbiol. 15:198.

Otte, E. 1963. Die heutigen ansichten über die atiologie der infetiösen bauchwassersucht der karpfen. Wien. Tierarztl. Monatsschr. 50:995-1005.

Rucker, R. R., B. J. Earp, and E. J. Ordal. 1954. Infectious diseases of Pacific salmon. Trans. Amer. Fish Soc. 83:297-312.

Somero, G. N., and A. L. DeVries. 1967. Temperature tolerance of some Antarctic fishes. Science. 156:257-8.

Stewart, J. E., and H. Rabin. 1970. Gaffkemia, a bacterial disease of lobsters (Genus Homarus). In A symposium on diseases of fishes and shellfishes (S. F. Snieszko, ed.). Amer. Fisheries Soc., Wash. D.C., p. 431-9.

Stokes, J. L. 1963. General biology and nomenclature of psychrophilic microorganisms. In Recent progress in microbiology (N.E. Gibbons, ed.). VIII Intern. Cong. Microbiol., Montreal, 1962. Univ. Toronto Press. Toronto. p. 187-92.

Stokes, J. L. 1968. Nature of psychrophilic microorganisms. In Low temperature biology of foodstuffs (J. Hawthorne, ed.). Pergamon Press. New York. p. 221-33.

Stokes, J. L., and M. L. Redmond. 1966. Quantitative ecology of psychrophilic microorganisms. Appl. Microbiol. 14:74-8.

Whitman, P. A., and R. T. Marshall. 1971. Characterization of two psychrophilic Pseudomonas bacteriophages isolated from ground beef. Appl. Microbiol. 22:463-8.

Wood, J. W. 1968. Diseases of Pacific salmon; their prevention and treatment. State of Wash. Dept. Fisheries Hatcheries Div.

Table 1. Genera of bacteria containing psychrophiles

Gram (+) Rods	Gram (-) Rods	Gram (+) Cocci
Actinomyces	Achromobacter	Micrococcus
Arthrobacter	Aerobacter	Sporosarcina
Bacillus	Aeromonas	Streptococcus
Clostridium	Alcaligenes	Gaffkya
Corynebacterium	Cytophaga	
Lactobacillus	Escherichia	
	Flavobacterium	
	Klebsiella	
	Moraxella	
	Proteus	
	Pseudomonas	
	Serratia	
	Vibrio	

Table 2. Fungal genera containing psychrophiles

Molds	Yeasts
Aspergillus	Blastomyces
Botrytis	Candida
Chaetostylum	Cryptococcus
Cladosporium	Mycotorula
Fusarium	Oidium
Monilia	Rhodotorula
Mucor	Saccharomyces
Oospora	Torula
Penicillium	Torulopsis
Phycomyces	
Sporotrychium	
Thamnidium	

Table 3. The incidence of psychrophilic bacteria in fresh water habitats

Sample	Percentage of the Total Population		
	Psychrophiles	Mesophiles	Thermophiles
Washington State			
Lake water	41-76	23-59	1
River water	16-29	73-84	1
Stream water	16-47	22-50	1
Lake mud	11-33	65-88	1
Louisiana State			
Lake water	15-31	69-85	0
River water	0	98-99	1-2
Lake mud	0-3	97-98	0-3
River mud	0	98	2-3

Data compiled from Stokes and Redmond, 1966; and Larkin, 1970.

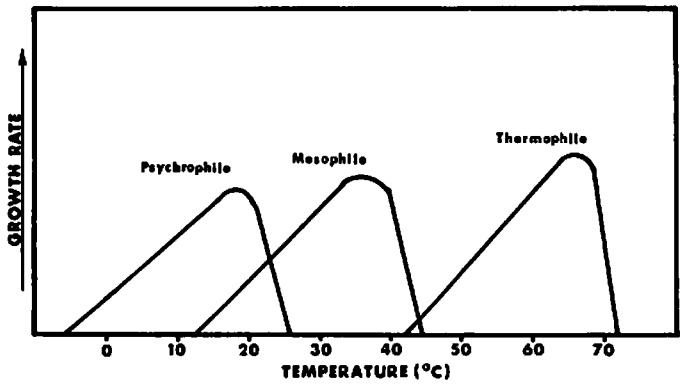

Figure 1. The relationship between a typical psychrophile, mesophile, and thermophile as regards their growth rate at various temperatures.

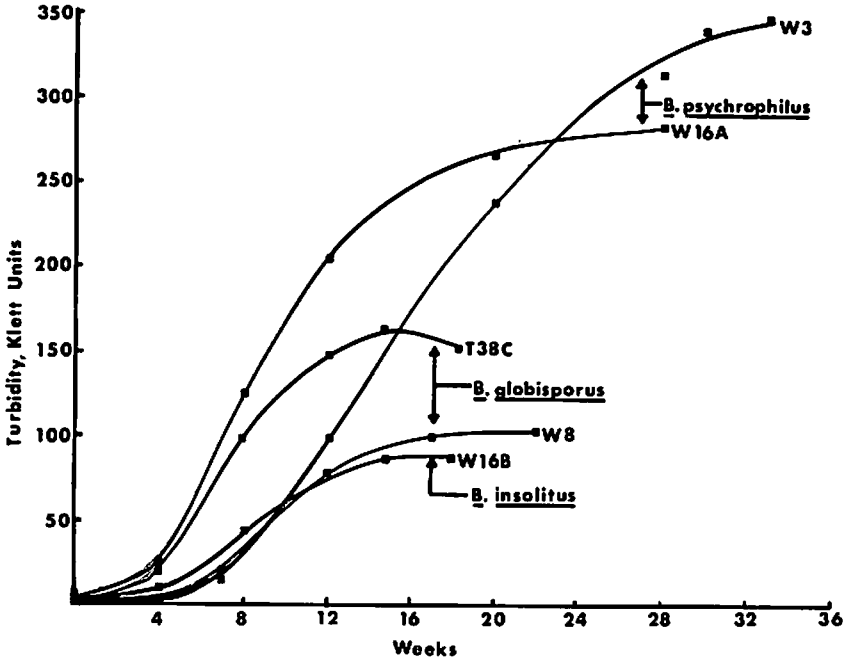

Figure 2. The growth of five strains of psychrophilic Bacillus at -5 to -7 C. Redrawn from Larkin and Stokes (1968).

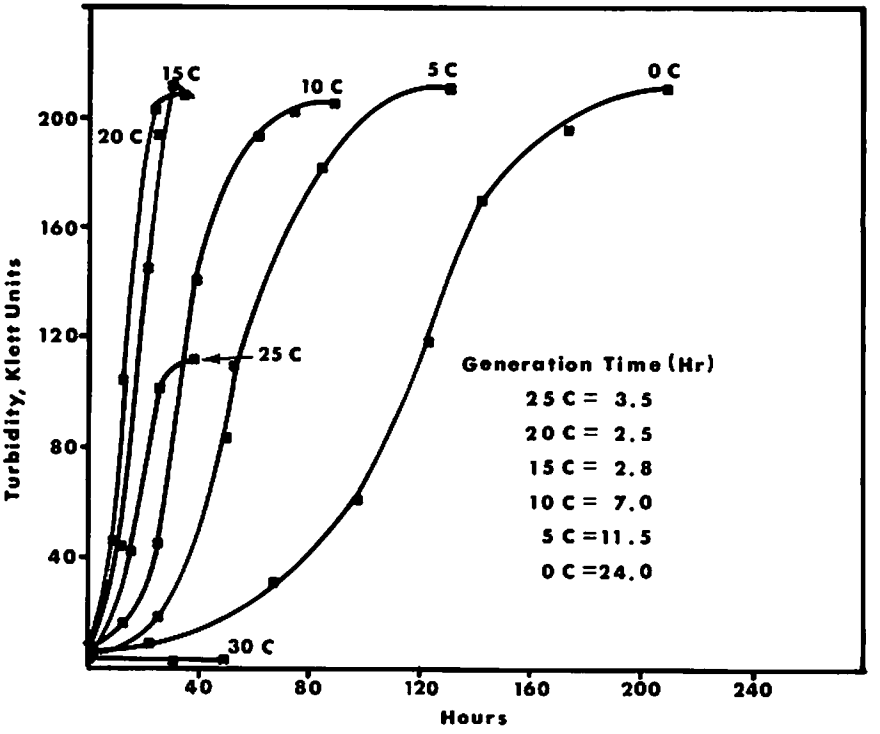

Figure 3. The effect of temperature on the growth of Bacillus insolitus W16B.

A SURVEY OF FUNGAL DISEASES OF MARINE ANIMALS WITH EMPHASIS ON RECENT RESEARCH CONCERNING *Lagenidium callinectes*[1]

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Of the more than 50,000 species of fungi known to man, only about 450-500 have been described as marine. Within this group, the number of fungi described as being pathogenic to marine animals is less than 25. This low incidence of described fungal pathogens does not necessarily indicate a scarcity of such organisms. Indeed, what is more likely indicated is the fact that there are relatively few investigators working in this area. However, this may not be the case for long, for as man expands his exploitation of marine organisms through farming of the sea, he will undoubtedly be faced repeatedly with the same adversaries that have plagued and at times thwarted man's attempts to farm the lands, i.e., fungi.

Table 1 lists some of the better known fungal parasites of marine animals and indicates their probable taxonomic placement within the recognized fungal groups. For information relative to these and other fungal diseases of marine organisms, see Johnson and Sparrow, 1961; Sindermann, 1970, 1974; and Snieszko, 1970.

The fungus, *Lagenidium callinectes*, was originally described by Couch (1942) from ova of the blue crab. Couch observed that approximately two to five percent of the eggs and zoea examined were infected with the fungus. Infected eggs would not hatch, and infected larvae failed to mature.

Since Couch's description, the most significant work on this fungus was done by Rogers-Talbert (1948) when she investigated the

[1] Research concerning *Lagenidium callinectes* was supported in part by NOAA Office of Sea Grant, Department of Commerce, under grant no. 2-35178, and the State of North Carolina, Department of Administration.

occurrence of L. callinectes in Chesapeake Bay. In this study, the fungus was observed to occur as a peripheral parasite of the crab's egg mass in up to 90 percent of a sample. Of these, about 14 percent of the crabs were found to be heavily infected with as many as 25 percent of the eggs of a single crab infected. It was further noted that transmission of the fungus was extremely rapid under laboratory conditions.

Since the work of Rogers-Talbert, Scott (1962), in a survey of the phycomycetous flora of marine and brackish waters around Gloucester Point, Virginia, isolated L. callinectes from 40 percent of the blue crab egg masses examined. Also Johnson and Bonner (1960) isolated L. callinectes from ova of the barnacle, Chelonibia patula. Fuller et al. (1964) isolated the same fungus from certain marine algae.

Cook (1971) attributed the extensive mortality of brown shrimp larvae occurring during the spring of 1971 at the Dow Chemical Co. Shrimp Hatchery in Freeport, Texas, to a marine species of the fungus Lagenidium. Recently, Lightner and Fontaine (1973) described a primary mycosis of white shrimp which they attributed to a species of Lagenidium. They reported that shrimp mortality in the experimental shrimp hatchery at the Galveston Laboratory of the NMFSL reached a level of 12.4 percent. In yet another situation, Mr. Bruce Hysmith, of Dow Chemical Company, Freeport, Texas, has reported sporadic mortality of up to 100 percent in 500 gallon rearing tanks containing larvae of P. aztecus (personal communication). Again, this mortality has been attributed to a fungus, probably a species of Lagenidium.

In North Carolina, our initial interest in L. callinectes concerned primarily its occurrence and distribution. Also of interest was the development and life history of the fungus.

With summer headquarters at the Duke University Marine Laboratory, Beaufort, N.C., extensive sampling for L. callinectes has been made in Bogue, Back, and Core Sounds and from the Newport and North River Estuaries.

Collective data over this period indicates marked fluctuations in the occurrence of L. callinectes from year to year. Infection has ranged from only six in 2,000 crabs examined during one season to a high in 1972 of 30 percent. The infection rate during the past summer was around three percent. There is no apparent correlation between infection rate and environmental factors. We can say, however, that where ovigerous crabs are plentiful there is a high incidence of infection. Our data indicate further that under favorable conditions this organism has the capacity for extremely high rates of infection and correspondingly a high destructive potential.

Certain results of our studies into the life history, development, and cytology of L. callinectes have been published previously (Amerson and Bland, 1973; Bland and Amerson, 1973a and 1973b) and will not be repeated here. Having completed these studies, however, investigations are now underway concerning possible means of control for L. callinectes and other fungal diseases of marine crustacea. Although our approach to this problem has been along several lines, our major efforts have been in the area of fungicidal control. Preliminary data concerning these studies are summarized in Table 2. Although only partially complete at this time, major portions of these studies concerning fungicidal control will be completed in the near future and will subsequently be made available through publication.

Our plans for future studies concerning L. callinectes and other fungal diseases of marine crustacea include:

1) A more comprehensive investigation into the occurrence and distribution of fungal diseases of marine crustacea with particular concern for diseases of potential candidates for aquaculture,

2) Studies into means of disease control other than highly toxic fungicides,

3) Field tests of those fungicides most suitable under laboratory conditions,

4) Investigations into the mode of action of the fungicides at the cellular level, and

5) Studies into the taxonomic relationships of certain fungi that have been isolated from various marine crustacea.

ACKNOWLEDGEMENTS

Research summarized here concerning Lagenidium callinectes was supported in part by NOAA of Sea Grant, Department of Commerce, under grant number 2-35178, and the State of North Carolina, Department of Administration. The U. S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon.

LITERATURE CITED

Amerson, H. V., and C. E. Bland. 1973. The occurrence of polycomplexes in the nucleus of encysting spores of Lagenidium callinectes, a marine phycomycete. Mycologia 65:966-70.

Bland, C. E., and H. V. Amerson. 1973a. Observations on Lagenidium callinectes Couch: isolation and sporangial development. Mycologia 65:310-20.

Bland, C. E., and H. V. Amerson. 1973b. Electron microscopy of zoosporogenesis in the marine phycomycete, Lagenidium callinectes Couch. Arch. Mikrobiol. 94:47-64.

Cook, H. L. 1971. Fungi parasitic on shrimp. FAO Aquacult. Bull. 3:13.

Couch, J. N. 1942. A new fungus on crab eggs. J. Elisha Mitchell Sci. Soc. 58:158-62.

Fuller, M. S., B. E. Fowles, and D. J. McLaughlin. 1964. Isolation and pure culture study of marine phycomycetes. Mycologia 56: 745-56.

Johnson, T. W., Jr., and R. R. Bonner. 1960. Lagenidium callinectes Couch in barnacle ova. J. Elisha Mitchell Sci. Soc. 76:147-9.

Johnson, T. W., Jr., and F. K. Sparrow, Jr. 1961. Fungi in oceans and estuaries. Hafner Co. New York. 668 p.

Lightner, D. V., and C. T. Fontaine. 1973. A new fungus disease of the white shrimp, Penaeus setiferus. J. Invert Pathol. 22:94-9.

Rogers-Talbert, R. 1948. The fungus Lagenidium callinectes Couch on eggs of the blue crab in Chesapeake Bay. Biol. Bull. (Woods Hole) 95:214-28.

Sandoz, M., R. Rogers, and C. L. Newcombe. 1944. Fungus infection of the eggs of the blue crab, Callinectes sapidus Rathbun. Science 99:124-5.

Scott, W. W. 1962. The aquatic phycomycetous flora of marine and brackish waters in the vicinity of Gloucester Point, Virginia. Virginia Inst. of Mar. Sci. (Gloucester Point) Rept. No. 36, 12.

Sindermann, C. J. 1970. Principal diseases of marine fishes and shellfish. Academic Press. New York. 369 p.

Sindermann, C. J. 1974. Handbook of diagnosis and control of diseases in mariculture. Informal Report No. 19. Middle Atlantic Coastal Fisheries Center. Oxford, Maryland. 174 p.

Scieszko, S. L. (ed). 1970. A symposium on diseases of fishes and shellfish. Spec. Pub. No. 5. Amer. Fish. Soc. Washington, D. C.

Table 1. Major fungal pathogens of marine animals

Major Taxonomic Group	Total Number of Marine Taxa	Pathogenic Genera	Hosts
Phycomycetes	50 (approximately)	Ichthyosporidium hoferi (Plehn & Mulsow) Pettit* Ichythophonus hoferi Plehn & Mulsow*	A variety of both marine and freshwater fishes
		Atkinsiella dubia (Atkins) Vishniac Plectospira dubia Atkins	Ova & larvae of the pea crab and several other marine invertebrates
		Pythium thalassium Atkins Pythium spp.	Ova of the pea crab and other invertebrates Shrimp
		Saprolegnia parasitica Coker	Larvae of Palaemonetes kadiakensis Atlantic salmon ?
		Leptolegnia marina Atkins	Ova & larvae of pea crab
		Spongiophaga communis Carter*	Various sponges
		Sirolpidium zoophthorum Vishniac	Larvae and juveniles of oysters and crabs
		Lagenidium callinectes Couch Lagenidium sp.	Ova and larvae of crabs, shrimp, and barnacles Internal organs of Lampanyctus ritteri
Ascomycetes	200 genera with over 300-400 species; one third are obligately marine	Trichosporon ladderi	Implicated in shrimp disease
Basidiomycetes	3	Unknown	
Organisms of uncertain Affinities			
Labyrinthulales	12 (approximately)	Labyrinthomyxa marina Mackin & Ray* (Dermocystidium marinum Mackin, Owen, and Collier)*	Bivalve molluscs

*Taxonomic placement uncertain

Table 2. Summary of preliminary data concerning studies of fungicidal control of Lagenidium callinectes

Fungicide	Minimum Lethal Concentration LC_{100} in ppm		Effect on Larval and Adult Invertebrates
	L-1 (isolated from C. sapidus)	L-3B (isolated from P. setiferus	
Benlate, 50% WP	29	39	Not tested
Captan, 50% WP	3.2	5.0	No effect on adult blue crab, lethal to blue crab larvae. Lethal to Penaeus azteeus at effective dosage** ***
Dichlone	10	21.0	Not tested
Difolatan, 39% flow	7.2	8.2	Not tested
Dyrene, 50% WP	6.0	21.0	Not tested
DS 9073*	1.3	3.2	Not tested
Manzate 200/ Dithane M-45 80% WP	2.1	2.9	No effect on adult blue crab, lethal to blue crab larvae. Lethal to Penaeus azteeus at effective dosage** ***
Terraclor, 75% WP	1.3	4.3	Studies underway
Tribasic Copper Sulfate	159	150	Not tested
Vitavax, 75% WP	37.5	38	Not tested
Malachite Green 99% pure	0.006	0.01	Studies underway
Treflan, 44-45% flow	1.5	NT	Studies underway

* An experimental fungicide/bactericide from ICI America, Inc.
** Tests conducted by Drs. J. Costlow, D. Lightner, or Mr. S. Hysmith.
*** When adult species of Murex, Uca, Arbacia, Astaria, Massarius obsoleta, and Terebra dislocata were placed in concentration of Captan and Manzate 200 lethal to L. callinectes, no adverse effect was observed during the 5-day exposure.

VIRAL DISEASES OF FISHES OF THE GULF OF MEXICO REGION

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INTRODUCTION

Fish virology as a science has existed for little more than 60 years, but most advancements in knowledge of virus diseases of fish have been made within the last 2 decades. Viruses of fish have been suspected since Weissenberg (1914) concluded that lymphocystis was caused by a virus; however, River's postulates for a fish virus were not fulfilled until infectious pancreatic necrosis (IPN) virus of trout was isolated in explant tissues (Wolf et al., 1960). Viruses thus far isolated from fish are morphologically, biochemically, and in most respects biologically similar to those from homoiotherms except that ichthic viruses replicate over a wide temperature range.

The majority of fish virus diseases occur in cultured species with high economic value diseases. Viral agents also cause acute diseases in ictalurids and carp. These diseases are principally confined to freshwater fish and anadromous salmonids, and virtually nothing is known about acute virus diseases of strictly marine species. Oncogenic virus associated diseases are common in wild, cultured, and aquarium marine and freshwater fish. With increased activity in mariculture, the possibility of discovering an acute viral agent in marine species will be greater. Possibly the proper environment for them to emerge has not been created as is the case with freshwater fish.

Several reviews of fish viruses are available (Nigrelli, 1952; Wolf, 1966). A conference sponsored jointly by the New York Academy of Science and the U.S. Fish and Wildlife Service in 1964 was devoted to virus diseases of poikilothermic vertibrates and approximately 60 percent of the papers were on viruses of fish (Whipple and Van Reym, 1965). There is also an excellent series of papers on the subject in A Symposium on Diseases of Fishes and Shellfishes (Snieszko, 1970).

Virus diseases which are of particular importance to the Gulf of Mexico and adjoining states will be discussed here, including diseases which are enzootic to the area and those which are most likely to be imported into the region in fish eggs or live fish. Salmonid viruses are included due to increased emphasis in raising trout in catfish ponds during the winter in the south and cage culture in the Gulf of Mexico (Tatum, 1973). A brief discussion on some of the problems involving fish virus research is also presented.

INFECTIOUS PANCREATIC NECROSIS VIRUS

Infectious pancreatic necrosis (IPN) virus disease of trout was first described by Wood et al. (1955), but Rivers' postulates were fulfilled for IPN by Wolf et al. (1960), which constitutes the first conclusive proof of a virus disease of fish. The virus causes an acute, virulent, highly contagious disease, that often results in a high percentage of mortality.

Clinical Signs

The clinical signs were described by Wood et al. (1955), Snieszko et al. (1957), and Wolf and Quimby (1967). IPN virus disease is marked by a rapid onset of mortality among susceptible trout. Fish infected with the disease usually have dark pigmentation, abdominal distention, and exophthalmia, and they often swim in a corkscrew motion on a horizontal plane. They then sink to the bottom where they respire weakly and die. Internally, infected fish have a pale liver, enlarged gall bladder, and hemorrhages in pancreatic tissue in the area of the pyloric cecae, and petechiae are present in organs throughout the visceral cavity. The intestine is void of food, but filled with a colorless fluid and at the juncture of the intestine and stomach there is a gelatinous plug which survives formalin preservation. The peritoneal cavity is usually filled with a clear yellowish fluid. The acinar cells of the pancreas are the first tissues in which histological lesions cevelop in experimentally infected fish, but other tissues become pathologically involved.

Morphology and Biophysical Nature

Characteristics of IPN virus were first reported by Malsberger and Cerini (1963), and the virus was initially classified as a picorna virus (Cerini and Malsberger, 1965). More recently it was reclassified as a rheovirus (Moss and Gravell, 1969). The virus is an icosohedral RNA particle (Figure 1) that measures 65 nm in diameter. It is stable in 50 percent glycerine at 4 C for over 5 years (Wolf et al., 1969). Also it remains infective for years when frozen at -20 C or lower and is very stable when lyophilized (Wolf et al., 1969; Cerini and Malsberger, 1965). The TRG-2 cell line is the culture system of choice for isolation of IPN virus.

Epizootiology

Fry or small fingerling trout are generally very susceptible, and as they become older they become more resistant to IPN virus. Brook trout (Salvelinus fontinalis) are probably the most susceptible species, but epizootics have occurred in rainbow trout (Salmo gairdneri), brown trout (S. trutta), and cutthroat trout (S. clarki). Atlantic salmon (S. salar) and coho salmon (Onchorhynchus kisutch) are susceptible, but other Pacific salmonids are refractive (Parisot et al., 1965; Mackelvie and Artsob, 1969; Wolf and Pettijon, 1970).

The mortality may start slowly, but after several days the death rate increases rapidly. Percentages of mortality may range from 1 to 74 percent, but this is partly governed by the age of the fish, species or strain of fish, virulence of the virus, and ambient water temperature (Wolf et al., 1961; Frantsi and Savan, 1971a). According to Frantsi and Savan (1971a), the percentage of mortality was reduced in susceptible size fish when water temperatures were depressed to 4.5 C. When the water temperature warmed to 15 C they had outgrown their susceptibility.

IPN virus has a wide geographical range. It occurs throughout the trout culture range in the U.S. and Canada and has been reported from Europe (Wolf and Quimby, 1971) and Japan (K. Wolf, personal communication). There is speculation that the disease was originally transported to these countries from the U.S. via infected eggs prior to presently used techniques for detecting infected populations.

Detection

Survivors of IPN epizootics may become carriers of the virus, periodically shedding virus via feces, eggs, or seminal fluids

throughout their adult life (Wolf and Quimby, 1967), and subsequently may transmit the disease vertically to their progeny via the reproductive products (Wolf et al., 1963). These carriers may be detected by isolating virus from physiological saline washes of the peritoneal cavity, fecal samples, ovarian fluids, or homogenized internal organs of fish which are suspect (Wolf, 1965; Wolf and Quimby, 1967, 1969; Wolf et al., 1968; Billi and Wolf, 1969; Frantsi and Savan, 1971b). Viral examination of internal organs is the most dependable technique, and using these IPN detection methods it is possible to demonstrate 5 to 30 percent of a carrier population to be positive at any one time. Once a carrier population is identified, these fish should be avoided as possible egg sources.

INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS

Infectious hematopoietic necrosis virus (IHNV) is the etiologic agent of a disease of sockeye salmon (O. nerka) and rainbow trout which was described by Amend et al. (1969). Earlier virus diseases of salmon of the Pacific northwest, such as the Sacramento River chinook salmon disease (Ross et al., 1960) and Oregon sockeye disease (Rucker et al., 1953), were probably caused by the same agent (Amend and Chambers, 1970a, 1970b). McCain et al. (1971) presented serologic evidence of an antigenic relationship between these two viruses.

Clinical Signs

Clinical signs of IHN were described by Amend et al. (1969); fish affected by the virus are lethargic, orienting in a vertical position, drifting aimlessly with the current, and occasionally expressing erratic swimming. Typically, the largest fish of a susceptible population are affected first. The earliest sign is the presence of an opaque fecal cast trailing from the anus, followed by exophthalmia, ascites, and appearance of hemorrhagic areas at the base of fins and throughout the musculature. Internally, tissues of infected fish are edematous; petechiae are present in the peritoneal wall, kidney, gas bladder, and lateral body walls; and some hemorrhagic enteritus develops. Usually the internal organs are pale. The peritoneal cavity is filled with a clear fluid, and the stomach and intestine contain clear mucus.

The first microscopic lesions of IHN diseased fish develop in hematopoietic tissues of the anterior kidney, but degeneration and necrosis is present in renal, kidney, spleen, and pancreas, and focal necrosis occurs in liver. A pathognomonic sign is the presence of a degeneration of granular cells in the wall of the alimentary canal (Yasutake, 1970). Pancreatic acinar and islet

cells may be in various stages of degeneration and necrosis.

Morphology and Biophysical Nature

IHNV is classified as a rhabdovirus by its bullet shaped morphology and approximate size of 65 to 100 nm wide by 120 nm long (Amend and Chambers, 1970a, 1970b; Darlington et al., 1972). IHN virus replicates in RTG-2 and FHM cell cultures at 10 to 18 C (Amend et al., 1969), but the lower temperature is nearer the optimum. The virus has been isolated from fish frozen at -20 C after seven months and from cell culture medium stored at 4 C for six months (Amend et al., 1969).

Epizootiology

Fry and fingerlings are most susceptible at which time the percentages of mortality may be 95 percent or greater, depending on the size of infected fish and ambient water temperature. The optimum temperature appears to be less than 16 C, and an epizootic can be brought under control by raising the water temperature to 18 C (Amend, 1970a). It is not known if surviving fish are carriers. Species susceptibility includes chinook salmon (O. tshawytischa), sockeye salmon, and rainbow trout.

IHNV has been primarily confined to the Pacific Northwest United States and southwestern Canada, but it has also been reported from Minnesota (Plumb, 1972a), Montana (Holloway and Smith, 1973), West Virginia, and Japan (Wolf et al., 1973).

Detection

IHNV may be detected in carrier populations only by virologically examining ovarian fluids from either pre-, post-, or actively-spawning females (Amend, 1970b). Sperm or tissue homogenates are not acceptable for determining IHN carrier fish.

CHANNEL CATFISH VIRUS

Channel catfish virus (CCV) is the etiologic agent of a highly contagious hemorrhagic disease of ictalurid fish. It is the only known herpesvirus isolated from fish. CCV was first reported by Fijan (1968) who, in the summer of that year, isolated a virus from dying channel catfish in four widely scattered epizootics and named the disease channel catfish virus disease (CCVD). Since the initial isolations, CCV has been found in over 30 epizootics in at least 11 states and one Central American country

(Plumb, 1971a; Plumb, unpublished data).

Clinical Signs

The clinical signs of CCV were described by Fijan et al. (1970) and Plumb (1971a). Fry and fingerling fish (Figure 2) are most susceptible. Typically infected fish are lethargic with hemorrhages in the skin and at the base of fins, exophthalmia usually in both eyes, distention of the abdominal cavity, pale gills, and dark pigmentation. Internally, the body cavity may be filled with a clear yellowish fluid; the liver has petechiae, but otherwise is pale; the kidney is enlarged and pale, but the spleen is deep red; and the stomach and intestine are very pale and void of food but have mucoid fluid. Some affected fish swim erratically and immediately prior to death may hang at the water surface in a head-up position, but this latter sign may be caused by other diseases of environmental disorders, especially when very young fish are involved.

Myxobacterial infections on the skin and fins frequently occur simultaneously with CCV infections which can confuse diagnosis of the disease. These bacterial infections may contribute to the death of infected fish, but the virus alone will cause a high rate of mortality.

Histologic findings of CCVD have been thus far described only from experimentally infected fish (Wolf et al., 1972; Plumb et al., 1974; Plumb and Gaines, in press). Fish which are diseased are hemorrhagic and edematous primarily within the internal organs. Liver epithelium has focal degeneration and necrosis, but renal hematopoietic and excretory tissues are necrotic and in some cases the later are nearly completely destroyed. Splenic white and red pulp are completely destroyed. Tissues of the digestive tract are edematous, and the mucosal layer of the intestine sloughs into the lumen. Skeletal muscle may be slightly hemorrhagic and edematous. These histopathologic signs agree with the gross clinical signs and also with levels of virus in various tissues reported by Plumb (1971b). Wolf (1973) reported, from personal communication of a co-worker, that material from a natural epizootic agreed for the most part with experimental findings.

After experimental infections of fingerlings, a viremia quickly develops (Plumb, 1971b; Plumb and Gaines, in press). Virus was isolated from the kidney, liver, intestine, and blood 24 hours after infection, but the kidney consistently produced the highest titers. Virus titers reached peaks at 72 to 96 hours after infection and then the level of virus subsided. These data, along with electron and light microscopy, indicate that the kidney is the primary organ of infection (Figure 3).

Morphology and Biophysical Nature

Channel catfish virus is a typical icosahedral herpesvirus with a diameter of 95-105 nm, but negatively stained preparations showed an enveloped particle with a diameter of 175-200 nm (Wolf and Darlington, 1971). The capsid is comprised of 162 hollow capsomeres. Electron microscopy of tissues from virus infected fish (Plumb, 1972b; Plumb et al., 1974) indicate that cellular changes and virus development *in vivo* are very similar to the *in vitro* cellular changes in BB cells reported by Wolf and Darlington (1971).

Based upon the morphology, nature of its cytopathic effect in cell cultures, and type of inclusion bodies, CCV is classified as a herpesvirus (Wolf and Darlington, 1971). Based on evidence presented by Wolf (1973) CCV is a DNA virus which is ether, chloroform, and glycerol labile. He also reported that the virus is very stable under frozen conditions; at -80 C no infectivity was lost after nine months and at -20 C infectivity persisted for four months (the virus carrier was not given). Plumb et al. (1973) reported that virus in infected fish stored at -80 C and -20 C survived for more than six months. Virus survived for at least 14 days in iced fish but survived for less than three days in specimens held at 22 C.

CCV is highly sensitive to drying (Table 1). The virus is killed on dried concrete chips in less than one day and on dried nylon netting or glass it survives for only 1.5 to 2 days. Infectivity is destroyed in less than one day in bottom muds of ponds. In water, however, survival is considerably longer (Figure 4); CCV remained infective in organically enriched pond water for less than four days at 25 C and for 26 days at 4 C, but in dechlorinated municipal water, infectivity was prolonged at both temperatures to approximately 11 days at 25 C and to more than 78 days at 4 C. Channel catfish virus would not survive very long in water or on equipment at the temperature one would expect the disease to occur, especially if facilities and equipment were dried.

Replication

Replication of CCV in BB cell cultures at 30 C was reported by Wolf and Darlington (1971). They also reported that CCV replicated only in BB cells, although 15 other lines were tested, including cell cultures from fish, amphibia, birds, and mammals. Wolf (1973) presented replication data in BB cells at temperatures 10 to 33 C, with the optimum being 30-33 C, but 37 C did not support replication.

Virus replication in cell culture can be detected by the

development of syncytia resulting from coalescing of many infected cells and inclusion of their nuclei. Prior to syncytial formation, cells become pyknotic and basophilic and show aggregation of chromatin, and lamellar-like intranuclear inclusion bodies appear. Karyorrhexis and karyolysis follow the appearance of syncytia.

Epizootiology

Channel catfish virus disease has generally been found in fry or young of the year fingerling channel catfish, although virus was isolated from one yearling group of five inch fingerlings. Younger fish are much more susceptible, and with increased age the percentage of mortality is less. In some cases 100 percent of infected fry have died, involving individual lots composed of 8,000 to 3 million fish. In the fingerling stage (up to 5-10 cm and less than five months old), mortality may exceed 90 percent, but lower mortalities have been reported. Size and age of fish alone do not determine the degree of mortality because environmental stresses such as low oxygen levels, high temperature, and crowding and the stress of handling or transporting infected fish may influence the final percentage. These factors may actually serve as triggering mechanisms for epizootics. Optimum temperature for epizootics is above 25 C, and mortality may occur at 20 C; below this temperature the effects of CCV are considerably less.

CCV is geographically confined for the most part to southern United States; however, in 1973, the virus was isolated from sick fish in California (W. H. Wingfield, personal communication), Nebraska, and one Central American country. Confirmed CCV epizootics have occurred in 11 states, and have involved fish from federal and state hatcheries as well as private industry.

The virus can be transmitted readily from infected fish to healthy fry or fingerlings by simply placing the healthy fish in the water outfall from infected stocks. The possibility of vertical transmission from carrier adult fish to their offspring exists, but the adult carrier state has not been demonstrated. There has been a tendency for epizootics to be associated with young fish of certain groups of broodfish, but the pattern of epizootic occurrences have been erratic enough to shed some doubt on the theory of a definite adult carrier.

There is no control of CCV, but Plumb (1973a) reduced the water temperature by 10 degrees C on infected fish 24 hours after infection and consequently reduced the mortality from 94 to 24 percent. Reduction of temperature at the onset of clinical signs and mortality reduced the mortality, but only from 68 to 78 percent.

The best means of combating CCVD is through avoidance of infected stocks. Detection of possible carriers is not possible through direct virus isolation, but there is some evidence that adult fish which were associated with epizootics had high levels of CCV specific antibody (Plumb, 1972b; Plumb, 1973b). This may possibly be used to detect potential sources of CCV.

CCV contaminated facilities and equipment can be disinfected by treating with 20 ppm of chlorine (Wolf, 1973) or by thoroughly drying the pond and equipment for several days.

LYMPHOCYSTIS

Lymphocystis virus disease is a chronic infection of many species of marine, freshwater, and aquarium fishes. It is the oldest and possibly the most common ichthic viral disease. Its history goes back a century (Lowe, 1874) when lymphocystis was reported from flounders in Europe. Weissenberg (1914) suggested a viral etiology and experimental transmission from diseased fish to non-infected fish was reported by Weissenberg (1939, 1945), Nigrelli (1952), and Wolf (1962). A viral etiology was not demonstrated until Wolf et al. (1965) did so by isolating the virus in tissue culture. The greatest importance of the disease is from an aesthetic point of view since the virus causes large, unsightly lesions on the skin and fins of infected fish, resulting in rejection from commercial and sport fish catches.

Clinical Signs and Cytology

Lymphocystis manifests itself as white or grayish papilloma-like lesions principally on the skin and fins. These lesions, with a vascular blood supply, are comprised of hypertrophied fibroblasts (Weissenberg, 1921). Nigrelli and Smith (1939) reported lesions on internal organs, and lesions of the viscera may be experimentally induced. Lawler and Dukes (1973) reported lymphocystis on the cornea of the eye of a marine fish. The histological nature of infected cells was described by Weissenberg (1949, 1951, 1960), Walker and Weissenberg (1965), Wolf (1966), and Christmas and Hawse (1970), and the cytological course of experimental lymphocystis was reported by Dunbar and Wolf (1966). Normal noninfected fibroblasts are 5-20 μ in diameter, but infected cells commonly attain a size of 100-500 μ in diameter and may even reach 5,000 μ. Infected cells possess an enlarged nucleus and Feulgen positive (Dunbar and Wolf, 1966) inclusion bodies (Walker and Weissenberg, 1965) in the cytoplasm that are thought to possibly be sites of virus replication. A PAS-positive (Howse and Christmas, 1970) hyaline membrane, approximately 10 μ thick,

surrounds the cell, and it is suggested that each individual infected cell produces its own membrane. These membranes may fuse in heavily infected fish, producing a gelatin-like matrix.

Morphology

The ultrastructure of lymphocystis virus was reported by Walker (1962), Walker and Weissenberg (1965), Walker and Wolf (1962), and Zwillenberg and Wolf (1967). These authors agree on the ultrastructure, size, and symmetry. Lymphocystis virus is a DNA, icosahedral virus and is classified as a myxovirus of approximately 300 nm (Figure 5). Zwillenberg and Wolf (1967) suggested that, based on their observations, it should be placed taxonomically close to Tipula iridescent virus of insects.

Epizootiology

Seldom, if ever, do fish die as a direct result of lymphocystis virus, althought thedisease may predispose fish to secondary bacterial infections which may be lethal. The percentage of lymphocystis infected fish in a population may vary from very low to 20-30 percent.

Source of virus is the infected host. As the lesions slough off or cells rupture, virus is released, but the exact mode of transmission to non-infected fish is not known. Experimentally, lymphocystis can be transmitted by transplantation of infected tissue in skin of another host by spraying virus on the gills or by co-habitation, but not by feeding infected material. Injury may be a predisposing factor in susceptibility (Olsen, 1958). Once the lesions are lost, the skin heals leaving no scar tissue to mark their presence. Lymphocystis is most frequently reported during winter when water temperatures are below 12 C, but it has also been reported during other times of the year.

A wide variety of fish species are susceptible to lymphocystis. The disease has been reported in five orders, 23 families, and 57 species (Nigrelli and Ruggieri, 1965; McCosker, 1969; McCosker and Nigrelli, 1971; Christmas and Howse, 1970; Lawler et al., 1974). The most common families infected are the Serranidae (basses), Centrarchidae (sunfishes), Percidae (perch), Cichlidae (cichlids), and Pleuronectidae (flounders). There is some evidence that a phylogenic relationship exists betweeen infectivity of virus from one group to another. The disease has been reported from North, Central, and South America; Europe; Atlantic, Pacific, and Indian Oceans; Gulf of Mexico; and Mediterranean and Arctic Seas. It is virtually world wide in distribution.

PROBLEMS IN FISH VIRUS RESEARCH

It has already been stated that intensive study of fish viruses is a relatively young science. This, along with the few scientists involved in fish virus research, has been a limiting factor in its advancement. Only in recent years have virologists from medical, veterinary, and academic disciplines joined in fish virus research with the fish virologists, who in many instances is a fishery biologist first and virologist second. Their welcomed interest will aid the advancement of fish virology.

The techniques required for fish cell culture, a vital segment of virus research were adapted from homoiothermic techniques. Until Wolf and Dunbar (1957) successfully established *in vitro* fish tissue culture techniques, followed by the first established ichthic cell line (Wolf and Quimby, 1962), little was achieved in the critical studies of fish virus research.

At least six certified ichthic cell lines are present in the American Type Culture Collection, the most notable of which are lines from rainbow trout (*Salmo gairdneri*) (RTG-2; CCL-55), fathead minnow (*Pimephales promelas*) (FHM; CCL-42), and the brown bullhead (*Ictalurus nebulosus*) (BB;CCL-59). The only certified cell line from marine species is that from the blue striped grunt (*Haemalon sciurns*) (GF;CCL-58). There are other non-certified lines that are in use among virologists, but there are no established lines from such groups as the pikes, basses, and shads or from the major marine groups. Lack of cell lines from these groups impedes fish virus research and should receive priority from fish tissue culturists.

The aquatic environment of the fish is one of the major problems with which the fish disease researcher must cope. Aside from the easily detected oncogenic virus diseases, very little is known about viruses of wild fish populations. Unlike the fish cultural environment where the host is readily observed, acute virus diseases are virtually unknown among fish in freshwater impoundments, rivers, and the marine environment. Fry and fingerling fish, generally considered to be highly susceptible to viruses, are seldom seen in the wild. A complete year class could be eliminated by a virus disease when they are in this stage of development, and their loss would not be realized for possibly years and only then through systematic population sampling. Therefore, the lack of methods to determine the presence or effect of acute virus diseases in wild fish limits virus research on such populations.

Detection of some of the known viruses of cultured fish is difficult. There is no problem in detecting IPN virus carrier fish or populations; however, detection of infectious hematopoietic

necrosis virus, spring viremia of carp, and channel catfish virus in the carrier state is either difficult or impossible. In acute conditions when fish are dying from these viruses, there is little difficulty in isolating and identifying the virus with virus specific antisera in appropriate cell culture systems. Therefore, if the epizootiology of these diseases is to be fully known, we must develop methods of detecting the carrier states of these potentially disasterous viruses.

Control of virus disease epizootics of fish is virtually nonexistent. Once a susceptible population becomes infected and mortality begins, little can be done to reduce the effects of the virus. Manipulating the water temperature of trout infected with IHN (Amend, 1970a) and channel catfish infected with CCV (Plumb, 1973a), may arrest the mortality, but practical methods of employing these techniques on a large scale have not been reported. The use of attenuated virus or vaccines to immunize fish against viral agents have not been widely explored. However, the use of the interferon producing ability of channel catfish against CCV (Gratzek, 1974) presents some intriguing possibilities.

LITERATURE CITED

Amend, D. F. 1970a. Control of infectious hematopoietic necrosis virus disease by elevating the water temperature. J. Fish. Res. Bd. of Canada. 27: 265-70.

Amend, D. F. 1970b. Approved procedure for determining absence of infectious hematopoietic necrosis (IHN) in salmonid fishes. Fur. Sport Fish. and Wildl. FDL 31:4 p.

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

Amend, D. F., and V. C. Chambers. 1970a. Morphology of certain viruses of salmonid fishes. I. In vitro studies of some viruses causing hematopoietic necrosis. J. Fish. Res. Bd. Canada. 27:1285-93.

Amend, D. F., and V. C. Chambers. 1970b. Morphology of certain viruses by salmonid fishes. II. In vivo studies of infectious hematopoietic necrosis virus. J. Fish. Res. Bd. Canada. 27: 1385-8.

Billi, J. L, and K. Wolf. 1969. Quantitative comparison of peritoneal washes and feces for detecting infectious pancreatic necrosis (IPN) virus in carrier brook trout. J. Fish. Res. Bd. Canada. 26:1459-65.

Cerini, C. P., and R. G. Malsberger. 1965. Morphology of infectious pancreatic necrosis virus. Ann. N.Y. Acad. Sci. 126: 315-9.

Christmas, J. Y., and H. D. Howse. 1970. The occurrence of lymphocystis in Micropogon undulatus and Cynoscion arenarius from Mississippi estuaries. Gulf. Res. Rep. 3:131-54.

Darlington, R. W., B. Trafford, and K. Wolf. 1972. Fish rhabdovirus: Morphology and ultrastructure of North American salmonids. Archiv. furdic gesamte Firusforschung. 39:256-64.

Dunbar, C. E., and K. Wolf. 1966. The cytological course of experimental lymphocystis in the bluegill. J. Infect. Dis. 116:466-72.

Fijan, N.N. 1968. Progress report on acute mortality of channel catfish fingerlings caused by a virus. Bull. Off. Int. Epiz. 69:1167-8.

Fijan, N. N., T. L. Wellborn, Jr., and J. P. Naftel. 1970. An acute viral disease of channel catfish. U. S. Depart. of Int. Bur. Sport Fish. and Wildl. Tech. Paper 43:11p.

Frantsi, C., and M. Savan. 1971a. Infectious pancreatic necrosis virus: Comparative frequencies of isolation from feces and organs of brook trout (Salvelinus fontinalis). J. Fish Res. Bd. Canada. 28:1064-5.

Frantsi, C., and M. Savan. 1971b. Infectious pancreatic necrosis virus: Comparitive frequencies of isolation from feces and organs of brook trout (Salvelinus fontinalis). J. Fish Res. Bd. Canada. 28:1064-5.

Gratzek, J. B. 1974. Antiviral resistance induced in channel catfish by polyinosinic polycytidylic acid (Poly I:C). Unpublished.

Gravell, M., and R. G. Malsberger. 1965. A permanent cell line from the fathead minnow (Pimephales promelas). Ann. N.Y. Acad. Sci. 126:555-65.

Holway, J. E., and C. E. Smith. 1973. Infectious hematopoietic necrosis of rainbow trout in Montana: A case report. J. Wildl. Dis. 9:287-90.

Howse, H. D., and J. Y. Christmas. 1970. Lymphocystic tumors: Histochemical identification of hyaline substances. Trans. Amer. Microsc. Soc. 89:276-82.

Lawler, A. R., and T. W. Dukes. 1973. Lymphocystis in the eye. The Vet. Rec. 93:297.

Lawler, A. R., H. D. Howse, and D. W. Cook. 1974. Silver perch, Bairdiella chrysura:New host for lymphocystis. Copia. 1974:266-9.

Lowe, J. 1874. Fauna and flora of Norfolk. Part IV. Trans Norfolk and Norwich Nat. Soc. p. 21-56.

MacKelvie, R. M., and H. Artsob. 1969. Infectious pancreatic necrosis virus in young salmonids of the Canadian Maritime provinces. J. Fish Res. Bd. Canada. 26:3259-62.

Malsberger, R. G., and C. P. Cerini. 1963. Characteristics of infectious pancreatic necrosis virus. J. Bact. 86:1283-7.

McCain, B. B., J. L. Fryer, and K. S. Pilcher. 1971. Antigenic relationship in a group of three viruses of salmonid fish by cross neutralization. Pro. Soc. Exp. Biol. Med. 137:1042-6.

McCosker, J. E. 1969. A behavioral correlate for the passage of lymphocystis disease in three blenoid fishes. Copeia. 1969: 636-7.

McCosker, J. E., and R. F. Nigrelli. 1971. New records of lymphocystis disease in four eastern Pacific fish species. J. Fish Res. Bd. Canada. 28:1809-10.

Moss, L. H., and M. Gravell. 1969. Ultrastructure and sequential development of infectious pancreatic necrosis virus. J. Virol. 3:52-8.

Nigrelli, R. F. 1952. Virus and tumors in fishes. Ann. N.Y. Acad. Sci. 54:1076-92.

Nigrelli, R. F., and G. D. Ruggieri. 1965. Studies on virus diseases of fishes. Spontaneous and experimentally induced cellular hypertrophy (lumphocystis disease) in fishes of the New York Aquarium, with a report of new cases and an annotated bibliography (1874-1965). Zoologica. 50:83-96.

Nigrelli, R. F., and G. M. Smith. 1939. Studies on lymphocystis disease in the orange file fish Ceratacanthus schoepfii (Walbaum) from Sandy Hook Bay. New Jersey Zool. 24:255-64.

Olsen, D. F. 1958. Statistics of a walleye sport fishery in a Minnesota lake. Trans. Amer. Fish Soc. 87:52-72.

Parisot, T.J., W. T. Yasutake, and G. W. Klontz. 1965. Virus diseases of the Salmonidae in western United States. I. Etiology and Epizootiology. Ann. N.Y. Acad. Sci. 126:502-19.

Plumb, J. A. 1971a. Channel catfish virus disease in southern United States. Proc. 25th Ann. Conf. Southe. Assoc. Game and Fish Comm. 25:489-93.

Plumb, J. A. 1971b. Tissue distribution of channel catfish virus. J. Wildl. Dis. 7:213-6.

Plumb, J. A. 1972a. A virus caused epizootic of rainbow trout (Salmo gairdneri) in Minnesota. Trans. Am. Fish. Soc. 101: 121-3.

Plumb, J. A. 1972b. Some biological aspects of channel catfish virus disease. Ph.D. Dissertation. Auburn University. Auburn, Alabama. 136 pp.

Plumb, J. A. 1973a. Effects of temperature change on mortality of fish infected with channel catfish virus. J. Fish. Res. Bd. Canada. 30:568-70.

Plumb, J. A. 1973b. Neutralization of channel catfish virus by serum of channel catfish. J. Wildl. Dis. 9:324-30.

Plumb, J. A., L. D. Wright, and V. L. Jones. 1973. Survival of channel catfish virus in chilled, frozen, and decomposing channel catfish. The Prog. Fish-Cult. 35:170-2.

Plumb, J. A. and J. L. Gaines. In Press. Channel catfish virus disease. In Symposium of fish pathology (B. G. Migaki and W. E. Ribelin, eds.). Armed Forces Inst. Path. U. of Wisconsin Press. Madison.

Plumb, J. A., J. L. Gaines, E. C. Mora, and G. B. Bradley. 1974. Histopathology and electron microscopy of channel catfish virus in infected channel catfish, Ictalurus punctatus (Rafinesque). J. Fish. Biol. (In Press).

Ross, A. J., J. Pelnar, and R. R. Rucker. 1960. A virus-like disease of chinook salmon. Trans. Amer. Fish. Soc. 89: 160-2.

Rucker, R. R., W. J. Whipple, J. R. Parvin, and C. A. Evans. 1953. A contagious disease of salmon, possibly of virus origin. U.S. Fish and Wildl. Serv. Fish Bull. No. 76. 46 pp.

Snieszko, S. F. 1970. A symposium on diseases of fishes and shellfishes. Amer. Fish. Soc. Spec. Pub. No. 5. 526 pp.

Snieszko, S. F., E. M. Wood, and W. T. Yasutake. 1957. Infectious pancreatic necrosis in trout. A.M.A. Arch. Path. 63: 229-33.

Tatum, W. 1973. Brackish water cage culture of rainbow trout (Salmo gairdneri), in South Alabama. Trans. Amer. Fish Soc. 102:826-8.

Walker, R. 1962. Fine structure of lymphocystis virus of fish. Virology. 18:503-5.

Walker, R., and R. Weissenburg. 1965. Conformity of light and electron microscopic studies on virus particles distribution in lymphocystis tumor cells of fish. Ann. N.Y. Acad. Sci. 126:375-85.

Walker, R., and K. Wolf. 1962. Virus array in lymphocystis cells of sunfish. Am. Zool. 2:566.

Weissenberg, R. 1914. Uber infetiose zellhypertrophie bei fischen (Lymphocystiserkrankung). Sits-Ber. Klg. Preuss. Akav. Wiss. 30:792-804.

Weissenberg, R. 1921. Lymphocystiskrankheit der fischei. In S.V. Prowozek and W. Noller, Handbuch der pathogen protozoen. 3:1344-3180.

Weissenberg, R. 1939. Studies on virus diseases of fish. III. Morphological and experimental observations on the lymphocystis disease of the pike perch, Stizostedion vitreum. Zoologica. 24:245-54.

Weissenberg, R. 1945. Studies on virus disease of fish. IV. Lymphocystis disease in Centrarchidae. Zoologica. 30:169-87.

Weissenberg, R. 1949. Studies on lymphocystis tumor cells of fish. I. Cancer Res. 9:537-42.

Weissenberg, R. 1951. Studies on lymphocystis tumor cells of fish. II. Cancer Res. 11:608-13.

Weissenberg, R. 1960. Some remarkable osmophilic structures of the inclusion bodies in the lymphocystis virus disease of the European flounder. Arch. Ges. Virusforsch. 10:253-63.

Whipple, H. E., and P. E. Van Reym. (Editors). 1965. Viral diseases of poikilothermic vertebrates. Ann. N.Y. Acad. Sci. 126:680 pp.

Wolf, K. 1962. Experimental propagation of lymphocystis disease of fishes. Virology. 18:249-56.

Wolf, K. 1965. Infectious pancreatic necrosis; Its detection and identification. The Prog. Fish Cult. 27:112.

Wolf, K. 1966. The fish viruses. In Advances in virus research. Academic Press (K.M. Smith and M.A. Lauffer, eds.) 12: 35-101.

Wolf, K. 1973. Herpesviruses of lower vertebrates. In The Herpes Viruses. Academic Press. p. 495-520.

Wolf, K. E., and R. W. Darlington. 1971. Channel catfish virus; a new herpesvirus of ictalurid fish. J. Virol. 8:525-33.

Wolf, K., and C. E. Dunbar. 1957. Cultivation of adult teleost tissues in vitro. Proc. Soc. Exptl. Biol. Med. 95:455-8.

Wolf, K., and L. L. Pettijohn. 1970. Infectious pancreatic necrosis virus isolated from coho salmon fingerlings. The Prog. Fish Cult. 32:17-8.

Wolf, K., and M. C. Quimby. 1962. Established eurythermic line of fish cells in vitro. Science. 135:1065-6.

Wolf, K., and M. C. Quimby. 1967. Infectious pancreatic necrosis (IPN): Its diagnosis, identification, detection and control. Rif. It. Pisc. Ittiopat. 11:76-84.

Wolf, K, and M. C. Quimby. 1969. Infectious pancreatic necrosis: Clinical and immune response of adult trouts to inoculation with live virus. J. Fish. Res. Bd. Canada. 26:2511-6.

Wolf, K., and M. C. Quimby. 1971. Salmonid viruses:Infectious pancreatic necrosis virus: morphology, pathology, and serology of first European isolations. Archiv. fur de Gesamte Virusforschung. 34:144-56.

Wolf, K., C. E. Dunbar, and E. A. Pyle. 1961. Infectious pancreatic necrosis of trout. II Experimental infections with brook trout. The Prog. Fish-Cult. 23:61-5.

Wolf, K., M. Gravell, and R. G. Malsberger. 1965. Lymphocystis virus: Isolation and propagation in centrarchid fish cell lines. Science. 151:1004-5.

Wolf, K., R. L. Herman, and C. P. Carlson. 1972. Fish viruses: Histopathologic changes associated with experimental channel catfish virus disease. J. Fish Res. Bd. Canada. 29:149-50.

Wolf, K., M. C. Quimby, and A. D. Bradford. 1963. Egg associated transmission of IPN virus of trouts. Virology. 21:317-21.

Wolf, K., M. C. Quimby, and C. P. Carlson. 1969. Infectious pancreatic necrosis virus; lyophilization and subsequent stability in storage at 4 C. Applied Micro. 17:623-4.

Wolf, K., M. C. Quimby, C. P. Carlson, and G. L. Bullock. 1968. Infectious pancreatic necrosis:Selection of virus free stock from a population of carrier trout. J. Fish. Res. Bd. Canada. 25:383-91.

Wolf, K., M. C. Quimby, L.L. Pettijohn, and M. L. Landolt. 1973. Fish viruses: Isolation and identification of infectious hematopoietic necrosis in eastern North America. J. Fish Res. Bd. Canada. 30:1625-7.

Wolf, K., S. F. Snieszko, C. E. Dunbar, and E. Pyle. 1960. Virus nature of infectious pancreatic necrosis in trout. Soc. Exp. Biol. and Med. 104:105-8.

Wood, E. M., S. F. Snieszko, and W. T. Yasutake. 1955. Infectious pancreatic necrosis in brook trout. A. M. A. Arch. Path. 60:26-8.

Yasutake, W. T. 1970. Comparative histopathology of epizootic salmonid virus disease. In A symposium on diseases of fishes and shellfishes (S. F. Snieszko, ed.). Amer. Fish. Soc. Spec. Publ. No. 5. pp 341-50.

Zwillenberg, L. O., and K. Wolf. 1967. Ultrastructure of lymphocystis virus. J. Virol. 2:393-9.

Table 1. Survival of CCV on dried materials and in pond mud at 25 C

Material	Days of Infectivity
Dried concrete chips	≤ 1
Dried nylon fish net	1.5-2
Dried glass (coverslip)	1.5-2
Pond mud	≤ 1

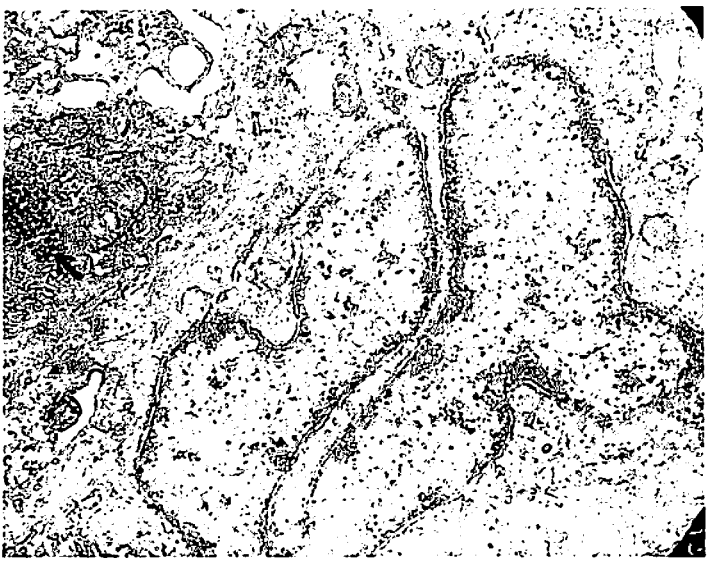

Figure 1. Infectious pancreatic necrosis virus in cytoplasm of RTG-2 cells (arrow). The virus size is 65 nm. (18,000X). (Photo courtesy of H. L. Moss III and M. Gravell through R. W. Darlington)

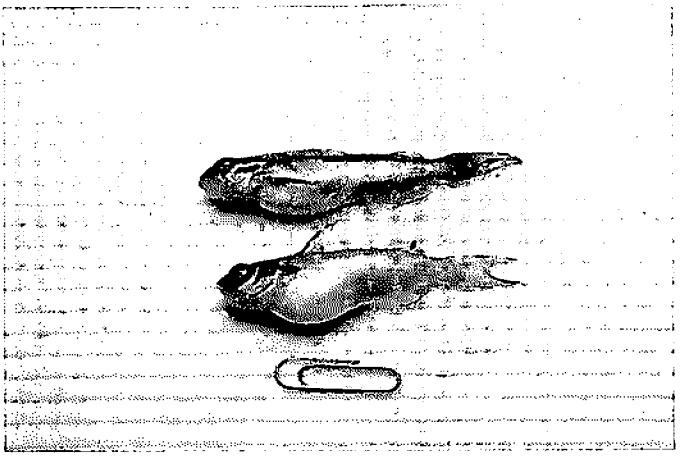

Figure 2. An 8 cm channel catfish infected with channel catfish virus. Note the abdominal distention (ascites) and exophthalmia.

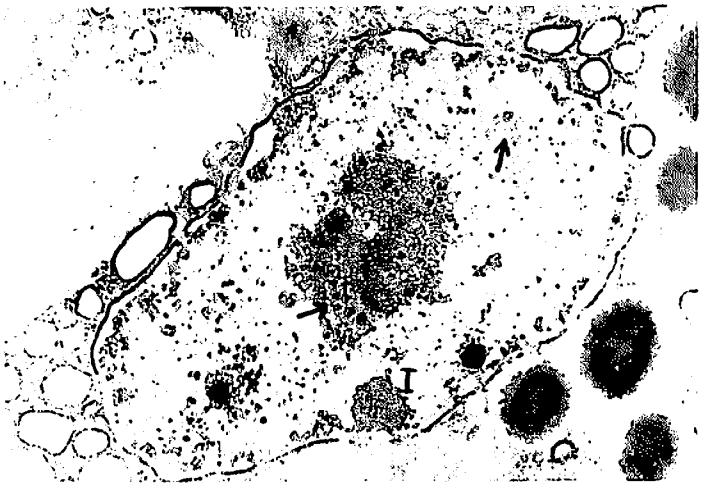

Figure 3. Channel catfish virus (arrows) within the nucleus of a spleen cell from a CCV infected channel catfish fingerling. Virion size is approximately 100 nm. Note laminar inclusion body (I). (20,500X)

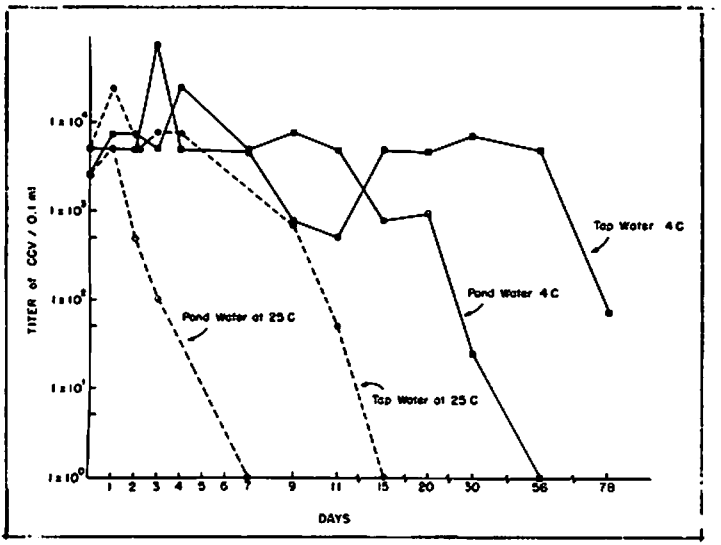

Figure 4. Survival of CCV in pond water and tap water held at 4 C and 25 C.

Figure 5. Lymphocystis virus in cytoplasm of epithelial cell from bluegill (Lepomis macrochirus) inoculated with virus 13 days earlier. The paler area (P) is the replicating pool of viral DNA with incomplete capsids at its border and fully formed virons (arrows) elsewhere. (15,000X) (Photo courtesy of R. Walker)

MICROSPORIDOSIS IN AQUATIC ANIMALS[1]

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The intensity of microsporidan epizootics has long been recognized in insects (Weiser, 1963). However, only during recent years has there been an increasing awareness of such infections within marine and fresh-water animals (Sindermann, 1970). Microsporidosis in food fish and commercially important decapod crustacea has received most of the attention.

Our studies have dealt primarily with microsporidosis in some of the food fishes of the Northwest Atlantic and Great Lakes and in commercially important decapod crustacea from the Gulf of Mexico.

Microsporidan parasites are slightly different from the standard eucaryotes because of their small size, absence of mitochondria, and development of a highly specialized infective spore stage. The spore has a complex internal structure designated as an extrusion apparatus (Weidner, 1972). Upon being ingested by an appropriate host, the extrusion apparatus discharges a fine membranous tube from the spore (Fig. 1). The tube is extruded with considerable force and serves as the vehicle by which the sporoplasm is inoculated into the host.

Microsporidosis has been devastating to a number of food fishes in the Northwest Atlantic. The angler fish, Lophius

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americanus, a large-sized, choice food of the orient and Western Europe, has had various difficulties becoming commercially established in the United States and Canada. It is believed that the principle factor limiting the abundance of this species is the remarkable nerve infection induced by Nosema lophii. An estimated 90 percent of the angler fish collected near Woods Hole, Mass. (summers, 1970-1973) had large colonies of N. lophii localized in greatly hypertrophied cell-bodies of giant sensory neurons in the cranial and spinal ganglia (Figs. 2 and 3). Cranial ganglia 5, 8, and 10 were consistently infected. Little scientific data are available on the effect of these parasites on angler fecundity and mortality, although there have been numerous reports by fishermen encountering beached anglers which had presumably succumbed to the infection.

Another potential food fish of the Northwest Atlantic is the ocean pout, Macrozoarces americanus. The high incidence of microsporidosis in pout is manifested as extensive intramuscular lesions which are particularly common in the anterior third of the musculature on either side of the spinal column (Sandholzer et al., 1945). The pout was introduced as a food fish in the early 1940's, and millions of pounds were marketed in New England. However, because of the wide incidence of a devastating microsporidan infection, the fishery quickly declined, and ocean pout completely disappeared from the market after 1944. Although the mode of transmission is not known, there are some indications that certain annelids which are part of the pout's food chain may serve as the source of infection.

Microsporidan infections have seriously affected two important food fish in the Northwest Atlantic, winter flounder, Pseudopleuronectes americanus, and smelt, Osmerus mordax. In both species, infections are localized as large colonies within the intestinal submucosa, mesenteries, and certain visceral organs. Infections cause extensive modifications in general structure and function of certain systems, thereby causing the inevitable acceleration in mortality rate (Stunkard and Lux, 1965).

An annual flounder epizootic peaks late each summer and may reach 70 percent of the estuarine young-of-the-year. Over past decades, the high incidence of Glugea hertwigi in smelt was believed to be the main reason for the decline of this fish to a negligible fishery in the Atlantic (Haley, 1952). Since the recent introduction of smelt to the Great Lakes, the G. hertwigi incidence has reached high levels in Lake Erie; indeed, this fishery is presently threatened.

The mode of transmission of microsporidosis is poorly understood for all affected fish species, including the commercially important food fish. The spore is presumed to be the infective

stage; however, feeding spores from infected to uninfected fish has not in the past resulted in transmission of the infection. Phase microscopy is adequate for observing whether spores are altered, unaffected, or empty after passage through an animal. In our studies of microsporidosis in winter flounder, G. stephani spores appeared unaltered after passage through flounder alimentary tracts. To follow this, populations of spores were placed in 1.5 cm millipore tubes (Fig. 4) with 0.45μ porosity and fed to four to six year-old flounder. After recovery of the chambers, the spores were viewed to determine whether they were empty, changed, or unaltered. Since G. stephani spores remained visibly unaltered during and after passage through flounder, we began testing Stunkard and Lux's (1965) idea that animals which are part of the fish's food chain may transfer the infection from fish to fish. The results of our experiments indicated that gammaridean amphipods caused up to 40 percent of the passaged G. stephani spores to become either altered or empty; furthermore, the longer the spores remained within the amphipod gut, the higher was the percentage of spores which were altered or empty. Hatching occurred primarily in the last part of the amphipod hindgut. There were no observable affects on spores which were passaged through the alimentary tracts of other groups of animals such as isopods (Ligia sp., Cyathura polita), polychaetes, and decapods (Homarus americanus, Carcinus meanus).

In a subsequent experiment, G. stephani (from flounder) and G. hertwigi (from commercially acquired smelt) spores were fed to separate populations of gammaridean amphipods. No parasite growth was observed in amphipods checked from each population on days one and five; however, by day 14 all the animals examined had vegetative and spore-forming stages localized in muscle tissue (Figs. 5, 6, 7, and 8). Although G. hertwigi and G. stephani produced similar pathology in host tissue (fish and amphipod), there were discernible differences in the spore stage. G. hertwigi, with a mean spore volume of $6.35\mu^3$ (Coulter Electronics, Inc.) was slightly smaller than G. stephani, which had a mean spore volume of $7.8\mu^3$. Moreover, G. hertwigi in amphipod muscle, had a more obvious pansporoblastic condition than G. stephani (see Figs. 5, 6, 7, and 8).

In nature, young-of-the-year flounder acquire microsporidosis after the fish grow to 40-50 mm. It is at this time that winter flounder become carnivorous on amphipod crustacea (Stunkard and Lux, 1965). Since transmission of microsporidosis has in the past proven largely unsuccessful in fish, a number of perplexing questions now arise: Are the gammaridean amphipods intermediate hosts which are required for the completion of the life cycle of the parasite from fish to fish? Are Glugea spores from amphipods different from those which develop in fish? Are Glugea hertwigi and G. stephani in fact crustacean microsporida which are annually displaced in fish such as flounder and smelt? If the last case is true, flounder and smelt microsporidosis could very well

disappear in the absence of infected amphipods.

Microsporidan epizootics have been reported in commercial decapod crustacea such as Penaeus setiferus, P. aztecus, P. duorarum, and Callinectes sapidus. Transmission of Nosema michaelis in blue crabs is direct (Sprague et al., 1968); however, feeding spores from infected to uninfected Penaeus has not in the past resulted in transmission of the infection. In fact, Thelohania spores appeared unaltered after passage through shrimp alimentary tracts.

Up to recently, much of the work on microsporida involved incidence, life cycles, pathology, and morphology. Physiological investigations were not done largely because laboratory controlled infections were not available on a continuing basis. This situation is beginning to change with the development of in vitro systems in the various laboratories (Ishihara and Sohi, 1966; Bismanis, 1970; Vavra et al., 1972; and Shadduck, 1969). Answers are urgently needed to determine the transmission capabilities for economically important microsporidan species and the nutritional requirements of the parasites and to develop simple diagnostic tests for microsporidan species identification.

LITERATURE CITED

Bismanis, J. E. 1970. Detection of latent murine nosematosis and growth of Nosema cuniculi in cell cultures. Canadian J. Microbiol. 16:237-42.

Haley, A. J. 1952. Preliminary observations on a severe epidemic of microsporidiosis in the smelt, Osmerus mordax (Mitchell). J. Parasit. 38:183.

Ishihara, R., and S. Sohi. 1966. Infection of ovarian tissue culture of Bombyx mori by Nosema bombycis spores. J. Invert. Path. 8:538-40.

Sandholzer, L. A., T. Nostrand, and L. Young. 1945. Studies on an Ichthyosporidian-like parasite of ocean pout (Zoarces anguillaris). Spec. Scient. Rep. Fish. U.S. Fish Wildl. Serv. No. 31. 12 p.

Shadduck, J. A. 1969. Nosema cuniculi: in vitro isolation. Science 166:516.

Sindermann, C. J. 1970. Principal diseases of marine fish and shellfish. Academic Press. New York.

Sprague, V., S. Vernick, and B. Lloyd, Jr. 1968. The fine structure of Nosema sp. Sprague 1965 (Microsporida, Nosematidae)

with particular reference to stages in sporogony. J. Invert. Path. 12:105-17.

Stunkard, H. W., and F. Lux. 1965. A microsporidian infection of the digestive tract of the winter flounder, Pseudopleuronectes americanus. Biol. Bull. 129:371-85.

Vavra, J., P. Bedrnik, and J. Cinatl. 1972. Isolation and in vitro cultivation of the mammalian microsporidian Encephalitozoon cuniculi. Folia Parasitologica (Praha) 19:349-54.

Weidner, E. 1972. Ultrastructural study of microsporidian invasion into cells. Z. Parasitenk. 40:227-42.

Weiser, J. 1963. Sporozoan infections. In Insect pathology: an advanced treatise (E. A. Steinhaus, ed.). Vol. 2. Academic Press. New York. p. 291-334.

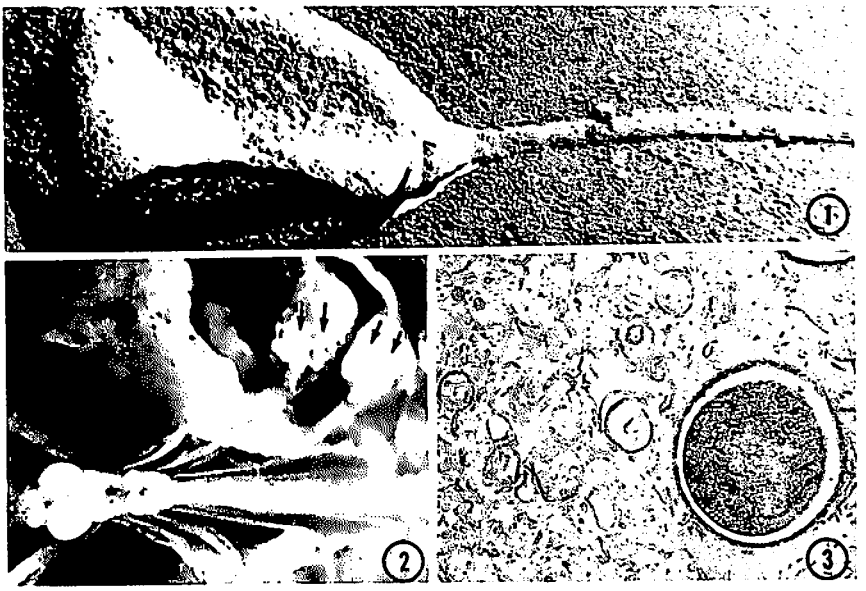

Figure 1. Carbon replica of discharged Nosema michaelis spore.

Figure 2. Actual size view of Nosema lophii infection clusters (note arrows) within several cranial ganglia of the angler fish, Lophius americanus.

Figure 3. Electron micrograph showing perimeter of spore colony within hypertrophied sensory neuron of a cranial ganglion of L. americanus.

Figure 4. Millipore chamber (2 mm x 1.5 cm) with 0.45 μ porosity. Spores are injected and the tube ends covered with envelope sealing wax.

Figure 5. Gammaridean amphipod muscle infection with G. hertwigi 14 days after feeding.

Figure 6. Gammaridean amphipod muscle infection with G. hertwigi 14 days after feeding. Arrow indicates one pansporoblastic cluster of spores.

Figure 7. Gammaridean amphipod muscle infection with G. stephani 14 days after feeding.

Figure 8. Gammaridean amphipod muscle infection with G. stephani 14 days after feeding. Arrow indicates large mitochondria external to spore clusters.

EFFECTS OF Gymnodinium breve RED TIDE ON FISHES AND BIRDS: A PRELIMINARY REPORT ON BEHAVIOR, ANATOMY, HEMATOLOGY, AND HISTOPATHOLOGY[1]

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INTRODUCTION

Previous Research

Red tides resulting from blooms of the toxic dinoflagellate Gymnodinium breve Davis occur along the west coast of Florida and elsewhere in the Gulf of Mexico. About every three to five years, severe blooms of this phytoplankter occur and are transported inshore causing massive lethal ichthyointoxication of many species. Great economic losses result, especially to the state's tourist industry.

Considerable study has been made of G. breve red tides, the causative organism, its toxin, and the resulting animal mortalities as summarized by Rounsefell and Nelson (1966) and Steidinger and Joyce (1973). Wilson and Collier (1955) and Ray and Wilson (1957) developed media to culture G. breve in the laboratory and

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was first to prove that this dinoflagellate does not produce a potent ichthyotoxin. Steidinger et al. (1973) have provided an excellent review of the chemical and physical characteristics of G. breve toxin(s) and its physiological effects. Numerous workers have repeatedly confirmed and emphasized neurotoxicity as the primary biological activity of this toxin. In vitro hemolytic activity has been detected in crude toxin extracts (Paster and Abbott, 1969; Martin et al., 1972; Martin and Padilla, 1971, 1973a, 1973b; Kim and Martin, 1974; and Kim et al., 1974) and in a purified nonneurotoxic fraction (Spiegelstein et al., 1973). Doig and Martin (1973) additionally demonstrated anticoagulant activity in toxin extracted from a natural G. breve red tide. They found this anticoagulant effect to be very mild and concluded, "It is evident from knowledge of death time that the ichthyotoxicity of gymnodin (the G. breve toxin) is not related to its anticoagulant properties." Spiegelstein et al. (1973) also summarized their findings stating, "More than 80 percent of the total activity of the crude toxin was a neurotoxin ..." These statements brought the chemists findings in line with those of biologists who had repeatedly documented the symptoms of neurointoxication preluding death in in vivo exposures to G. breve red tides or cultures.

As early as 1882, Glazier (1882) reported that fish captive in on-board live wells with pass-through water circulation from the outside died quickly when the vessel moved through discolored "poisoned" water. The same year Moore reported observing rapid death when free swimming fish encountered such discolored patches of red tide water. Glennan (1887) once again reported sudden deaths of live cargos of fish being held in live wells when the vessels passed through strips of discolored water. Galtsoff (1948) and Rounsefell and Nelson (1964) reviewed these and other reports of sudden fish death upon exposure to visible red tide and some of the accompanying neurointoxication-like signs.

Ray and Wilson (1957) conclusively demonstrated a complex lethal neurointoxicative action on several fishes with death finally resulting from respiratory arrest. Numerous laboratory animals have subsequently been used to bioassay for G. breve toxin, and signs of neurointoxication have been uniformly observed in all susceptible invertebrates, fishes, amphibians, birds, and mammals tested (c.f. Treiff et al., 1973; Sievers, 1969; Steidinger et al., 1973).

Humans are infrequently affected by G. breve toxin due to ingestion of shellfish that have become toxic through natural exposures (McFarren et al., 1965; Cummins and Stevens, 1970; Hemmert, 1974, unpublished data). The symptomology in these cases has been complex, somewhat variable, and usually mild but has been indisputably typical of neuromuscular intoxication.

As a result of numerous and varied field and laboratory studies; mortalities resulting directly from G. breve red tides have been accepted to be of strictly neurotoxic etiology. The apodictical laboratory results on vertebrates (and even most invetebrates) have derived from short term -- usually single dose -- exposures to toxin. Most deaths have been observed in less than six hours post exposure, with experimental animals living at termination being considered "survivors." The majority of tests can thus be considered demonstrative of acute intoxication. Even most reported field observations have been indicative that sudden exposures to concentrated red tide result in acute signs and rapid death.

It is possible that many, if not most, mortalities resulting from red tides are not acute but occur only after days or weeks of exposure to subacute toxin concentrations. Mortalities typically occur throughout the course of a red tide, even in places where significant to lethal concentrations of G. breve (5000 to 250,000 or more cells per liter) have been continually present for days and weeks. Such red tide may occupy areas of hundreds of square kilometers (Steidinger and Ingle, 1972; Steidinger, 1973). It is unlikely that rapid immigrations from outside the red tide area account for significant proportions of the mortalities, particularly since most organisms typically killed are sedentary or even sessile species, i.e. sheepshead, burrowing eels, blow fishes, toadfishes, batfishes, gastropods, and tube building polychaete worms.

In surveying the literature, we could find no reliable experimental data on long term exposures of vertebrates (more than 48 hrs) and no evidence of any hematologic, necroptic, or histopathologic examinations of any animal affected or killed by G. breve. Such a study could be extremely valuable if it:

1) Elucidates the mechanism of toxic action in fishes and other animals (including humans),

2) Indicates how some species resist intoxication more than others,

3) Leads to development of a simpler, more sensitive assay for G. breve toxin,

4) Makes diagnosis of red tide intoxication possible, or

5) Provides ET_{50}/ LD_{50} data on various species that, combined with information on G. breve concentrations and fishery data, could be used to predict location, severity, and specification of fish kills.

The 1973-74 Red Tide

The recent G. breve red tide along the west coast of Florida provided an excellent opportunity to collect and analyze red tide mortalities. The first fish kills were reported offshore in late October, 1973, and distressed tomtates (Haemulon aurolineatum) were collected for pathological examination. Sporadic fish kills were observed into early December, 1973, primarily 11-40 km (7-25 mi) offshore from St. Petersburg to Sarasota and from Boca Grande to south Sanibel Island with G. breve counts generally between 2,000 to 1.6 million cells per liter but sporadically higher (250,000 or more cells per litter is generally considered lethal to fish) (Figure 1). This red tide then subsided.

After about a month's absence, fish kills were again reported in January, 1974, inshore near Sarasota, Clearwater, and other areas. These kills were almost monospecifically striped mullet, Mugil cephalus. Mullet fishermen were working these areas intensely to catch the spawning run and, as a result, were blamed for the dead fish. We collected fish for examination to determine actual cause of death. The absence of net scars or other capture markings, the presence of G. breve in the water (90,000 to 330,000 cells per liter), plus hematological abnormalities and pathology similar to the tomtates indicated that most, if not all, the mullet were killed by G. breve red tide. The monospecific nature of these kills probably resulted from the high sensitivity of this animal to red tide plus its dense nearshore spawning aggregations.

This red tide continued somewhat sporadically along the west coast of Florida in nearshore waters from Charlotte Harbor north to Tarpon Springs, a linear distance of some 190 km (120 mi). Counts generally remained low to moderate, and fish kills were scattered. In February, however, the red tide entered and began to concentrate in Boca Ciega Bay and Tampa Bay. Counts of G. breve were commonly near two million cells per liter and locally higher (more than 50 million cells per liter). Massive and continual fish kills resulted, affording ample opportunity for observation and collection of distressed fishes. This red tide abated through April and by May, 1974, was confined to a few small areas from Tarpon Springs to Charlotte Harbor.

During the last week of February, cormorants, mergansers, and lesser scaup ducks (Aythya affinis) were reported dying in red tide areas. The former two groups responded well to antibiotic therapy (chloramphenical and Furacin) (Heath and Albers, pers. comm.) thus indicating a disease of bacterial or other susceptible etiology. Lesser scaup did not respond. Deaths among the former two species soon ceased but lesser scaup continued to die in

increasing numbers. The red tide was blamed by lay observers for the scaup deaths. As a result, we ran pathologies on both sick and healthy birds in an attempt to determine cause of death and possible involvement of red tide. Meanwhile, the ducks continued to die in various areas with the deaths finally becoming concentrated in Hillsborough Bay at Tampa where red tide had been absent or minor. Reports of bird mortalities dwindled as the flocks migrated northward in early April toward their breeding areas in Canada. However, lesser scaup mortalities were reported from Mississippi and other flyway refuges (Overstreet, pers. comm).

METHODS AND MATERIALS

During this red tide, 177 affected organisms or species lots were collected for detailed pathologic analysis. Of these, 124 fishes and 24 lesser scaup ducks were subjected to necroptic examination. The remaining 29 cases were invertebrate species lots most of which were subject to histopathological analyses only. Affected organisms were compared subjectively and objectively with a collection of over 500 case histories and 7,000 slides on file at the Marine Research Laboratory in order to define abnormalities. Norms from the literature, such as Magath and Higgins, 1934; Lucas and Jamroz, 1961; Sinderman, 1961, 1967; Saunders, 1966; Ward, 1969; Brunori et al., 1973; Ward and Ezell, 1967; Ward and Davis, 1974; and Kocan, 1972, were also consulted for comparison.

Methods varied in detail from one case to the next, depending mostly on the type of specimen (fish or bird), its size, and its condition. The following procedures, therefore, represent the handling of most, but not all, specimens.

Distressed fishes and most distressed ducks were collected from red tide areas, and all were captured by hand or by dip net. Some freshly dead fishes and ducks, prerigor or in rigor mortis, were collected by hand from the water surface or shoreline areas. Water samples were taken at collection sites for G. breve enumeration. Ducks with normal behavior and appearance were killed (rifle or shotgun) for use as "control" specimens. Other ducks were trapped well outside the red tide area in the St. Marks Wildlife Refuge for use as controls. Several diseased ducks remained alive in the laboratory and signs were noted including body temperature (rectal or underwing); respiration rate; heart rate; motor control; balance, blinking, righting, and other reflexes; iridic response to light; nasal, oral, and ocular discharges; defecation; drinking; and general activity.

All animals were transported to the laboratory unrefrigerated as rapidly as possible and necropsied immediately. Most

necropsies were begun within 45 minutes of collection or death and none after more than six hours.

Standard length and weight measurements were taken on each animal and notes made on external appearance of the fishes including color, ectoparasites, sliminess, external lesions, fecal extrusion, pupillary cloudiness, rigor mortis, fin condition, nares, bucal area, gill color, parasites, lesions, lamellar structure and body texture, shape, and odor. Similar notes were made on the ducks as applicable plus notes of molting status and any ocular, oral, or nasal discharge. Gills and skin were subjected to stereomicroscope examination. Scrapings of gill lamellae and skin were examined as wet mounts under a compound microscope for bacterial abundance and morphotypes, parasites, hemorrahage, branchial lamellae abnormalities, edema, and sloughing.

In fishes, blood samples were taken from one of the subcephalic or cervical sinuses, usually the common cardinal, using a nonheparinized and then a heparinized syringe on the same needle. Intracardial blood was taken from the birds via right mediolateral entry using the same double syringe technique. A generalized whole blood clotting time (WBCT) was obtained from a pool placed in a well of a three well spot plate. A wet mount of diluted blood was made and observed at 700x or more phase contrast for erythrocyte cytology, density, vacuolation, morphology (e.g., edge type, flatness), nuclear appearance, placement, and size. Similar observations were made of thrombocytes and other types of leucocytes for fibrin formation, contraction, and erythrocyte and achromic normoblast (pronormoblast or erythroblast) adhesion and for plasma debris, hemoparasites, and bacteria. Observations were made of whole blood color, appearance, optical density (opacity), viscosity, and type of clotting (e.g., hard, soft, incomplete, nonretractile, dark, lithy). Smears of unheparinized blood were made, air dried, and heat and methanol fixed. These were stained by Wrights, CamkoQuik, Giemsa, Perls iron, and MacCallum-Goodpasture techniques. Two hematocrit tubes were filled and centrifuged (1,000 x G, 15 min) for packed cell volume (PCV). Note was made of any white layer (leucocytes and pronormoblasts) that formed and for presence of manifest plasma hemoglobin and plasma color. The hematocrit tube plasma was expressed directly onto test strips (Hemastix) and quantitated colometrically for protein, glucose, pH, and occult hemoglobin. O ly the top half of the serum column of each tube was utilized, being expressed by insertion of a close fitting metal plunger in the opposite end. Pipette (RBC) dilutions of 100:1 and/or 200:1 were made and counted on a hematocytometer for erythrocytes, normoblasts, leucocytes, thrombocytes, hemozoa (if present), and bacteria (if present), with subjective note of cell distribution, uniformity, and absence or degree at clumping. Samples were prepared and stored for later standard colometric cyanomethemoglobin determination.

If 1.5 cc or more additional blood could be withdrawn after the above tests, it was centrifuged at 1000 x G for 15 minutes and the pellet and serum separated and frozen for more detailed chemical analysis, the results of which will be reported later.

Fish dissections were begun by resection of a rectangular portion of the epaxial muscle masses adjacent to the origin of the dorsal fin and including the overlying intact integument. A comparable muscle/epithelium cubic sample was taken of the medioventral pectoralis in the birds. In all cases, a small transverse slice of this tissue was removed and fixed in cacodylate or phosphate-buffered 2.5 percent gluteraldehyde and the remainder fixed in Davidson's fixative. These dual gluteraldehyde and Davidson's fixations were used routinely throughout this study. Several arches of the left gill and the left pectoral fin were also removed and fixed. Fishes were opened by removal of the left abdominal cavity wall and without entry of the perianal area. In birds, the entire sternum, including adjacent thoracic ribs and tissues, was resectioned to expose the internal organs. In all cases, the perianal area was circumscribed and later resectioned with the intestium crassum. Internal organs were sampled for microbiological examination; photographed; noted as to color, size, turgor, gross appearance, position, parasites, and any gross pathology; and then individually removed, weighed, and fixed. Due to their fragility, bird lungs were removed while still attached to the thoracic ribs and vertebrae and then vaccuum infiltrated with fixatives. Pleural tissue was separated for ultrastructural and histological processing after fixation. In both fish and birds, the cranial cap (frontal, supraoccipital area) was then carefully removed to expose the brain and anterior spinal cord. Descriptive notes were made; the cranial and spinal nerve pairs were severed; and the brain was removed, weighed, and fixed. The entire left ocular cavity with oculus in situ and then the left ethmoid and nares were removed and fixed. Additionally, the left salt gland of the ducks was removed and fixed.

Table 1 lists the organs removed and the usual sectioning plane and staining schedules. All gluteraldehyde samples were fixed at 3 C for 6 to 24 hrs, depending on the size of the tissue samples, and then placed in buffer and returned to refrigerated storage for later preparation for electron microscopic examination. All tissues in Davidson's fixative were removed after 24 to 48 hrs and placed in 70 percent ethanol for storage. Standard histological preparation techniques followed for subsequent light microscope examination.

RESULTS

Ichthyopathology

The data presented below derives from pathologic examination of 124 fishes, 45 of which were subjected to the complete necroptic procedure including detailed hematological workup. A total of 13 species of 13 genera membering among 11 families in 6 orders and 2 classes were studied, but most work was done on the few more common species (e.g., striped mullet, Mugil cephalus, 19 specimens) for which more background data was available. Although most of these fishes were adults, a range from adults to smaller sizes of some species was examined, e.g., mullet and ladyfish (Elops saurus). General findings were fairly consistent, but there was much quantitative interspecific variation. Expected intraspecific variation was also present.

The following summarizes the abnormalities repeatedly seen in fishes killed in red tide areas: 1) anoxic predeath behavior, 2) cyanemia, 3) severe normoblastosis (achromic), 4) moderate to severe normochromic normacytic anemia, 5) markedly increased blood viscosity, 6) increased WBCT, 7) increased PVC, 8) increased total blood count (TBC), 9) increased mean cell volume (MCV), 10) thromobocytopenia, 11) leucocytopenia, 12) plasma debris, 13) elevated manifest plasma hemoglobin, 14) absent or reduced recent feeding, 15) splenomegally, and 16) hemosiderin deposition in the hepatic vascular endothelium.

Toxicological sign complex

Distressed fishes were observed on several occasions in areas of low to moderate G. breve concentrations (greater than 250,000 cells per liter). Mullet repeatedly demonstrated behavior almost identical to that seen under conditions of severe anoxia. They came to the surface swimming slowly and displaying gulping activity. Individuals would slow their movement and sink slowly, frequently losing equilibrium and even tumbling. They would then return feebly to the surface, repeating these actions numerous times but with decreasing vigor until all equilibrium and swimming ability was lost and death ensued, 15-30 min after initiation of distress behavior. About 70 percent of the fish sank upon death, while the remainder floated. Typically, less than 10 percent of the fish in a school displayed this terminal distress at any one time. The remainder behaved normally except for a depressed irritability as demonstrated by failure to flee until approached very closely and by inability to avoid a hand-held dip net. Once initiated, the fright response appeared normal. Distinct hyperactivity was not seen in these fish; however, those sinking from the surface frequently showed an uncoordinated

writhing and tumbling, apparently in futile attempts to regain their equilibrium. Another observer (L. Tester, pers. comm.) also recorded an almost identical sign complex in mullet but at the much higher G. breve concentration of two to five million cells per liter. Death seemed to occur more quickly, usually four to six minutes after initiation of distress activity. Similar activity depression and anoxia-like signs were also seen in tomtates, orange filefish (Ceratacanthus schoepfii) (F. Smith, pers. comm.), and goldfish (Carassius auratus).

Ladyfish and small bay anchovies (Anchoa mitchelli) showed distress signs distinctly different from those described above. Both species swam very rapidly and erratically with the body appearing to quiver. They moved in a random pattern, alternately striking the surface and floundering there momentarily before sounding. Low, uncoordinated jumps were common, even in the anchovy, a fish that seldom jumps from the water. Death in both species seemed to overtake them rather suddenly, with movement, including gill movement, slowing and ceasing, followed by cardiac arrest in 15 to 30 sec. Episodes of such neurointoxication-like behavior occurred in individual fish of a large school of ladyfish in a single small bayou continually for over four days. The same phenomenon occurred in a large school of anchovies in a larger bayou for over two days. In both cases, individual fishes seemed to become affected quite suddenly and die in a relatively short time, less than 15 min in the case of the anchovies. Other members of the school showed normal behavior. Since Starr (1958) observed signs of neurointoxication in mullet, this fish may show two different sign complexes, anoxic and neurointoxicative, under different exposure conditions, such as a gradually increasing low level in contrast to a sudden high toxin level (high G. breve count) in an artificial environment.

Redfish (Scienops ocellatus), sheepshead (Archeosargus probatocephalus), pinfish (Lagodon rhomboides), and spotted sea trout (Cynoscion nebulosus) were captured near Crystal River, Florida, far from the red tide area, and maintained for four weeks in tanks on a flow-through sea water system from Bayboro Harbor off Tampa Bay. Concentration of G. breve near the intake averaged about 300,000 cells per liter, ranging from 2,000 to 1.6 million cells per liter during the four week test. The sea trout showed predeath behavior similar to that seen in mullet. The swimming ability seemed to be impaired by a change from the coordinated anterior to posterior sinusoidal wave motion to a simple arc-like flexation to alternate sides. Neither oxygen deficiency nor handling injury can be excluded as causes of this anoxic type behavior and death. Redfish in these same tanks showed no distress during this test, with the one death resulting from accidental entanglement in the netting over the tanks. No distress behavior was observed in pinfish or sheepshead in these tanks.

In tests designed to evaluate methods of detoxifying red tide water with O_3 and Cl_2 (Blogoslawski, pers. comm.; Moe, pers. comm.), water was collected from an area where bay anchovies were acting normally (count 1.1 million cells per liter) and was placed in five liter vessels. Young two cm TL, laboratory reared Pacific clownfish, Amphiprion ocellarus, were then introduced. Death occurred in 15 to 135 min in all exposed fish and was accompanied by a sign complex possibly indicative of neurointoxication. Normal activity was slowly depressed, and the animals began to rest on the bottom, swimming periodically to the surface and drifting downward again. Fishes began to list increasingly to one side while on the bottom, and there was a tendency toward inflexible curvature of the body axis making swimming increasingly more difficult. Some fish slowly drifted over on their side and died quietly while others displayed a 30 sec to five min period of uncoordinated mild hyperactivity near the surface prior to death. This common aquarium fish endemic to the Indo-Pacific (never naturally exposed to G. breve toxin) was sensitive to acute intoxication in these tests and may be an effective bioassay tool.

Hematology

Overt cyanemia was present in 66 percent of all fish examined and in 84 percent of the mullet (Table 2). Diagnosis was made visually as a distinct bluish coloration of blood samples immediately upon withdrawal before exposure to air. No difference was seen in heparinized and nonheparininzed samples and the discoloration did not subside upon exposure to air. In many of these fishes, cyanemia was so marked as to be externally detectable as a bluish or purple discoloration of the gills. The blood appeared unusually dark in many fish not qualitatively classified as cyanemic (no spectroscopic quantification was attempted). Overt cyanemia was not evident in spotted seatrout and ladyfish.

Whole blood hyperviscosity was manifest in over 50 percent of the fishes for which data was taken (85 percent of necropsies, Table 2). Of the mullet, almost 70 percent showed hyperviscosity. Viscosity increases were noted as distinctly larger drops from syringes, higher menisci on glass slides, and a resistance to linear gravity flow on tilted surfaces. No quantitative measurements were made due to the unavailability of a viscosometer. In many cases, viscosity of uncoagulated blood appeared comparable to that of machine oil (greater than 35 centipoises). We subjectively questioned how the heart could maintain blood circulation against the substantial back pressure that must have resulted from passage of such viscous blood through the vascular system.

In most cases, WBCT was greater than normal for teleost fishes even though it was quite variable, both inter- and

intraspecifically (Table 2). Although exact clotting times are uncertain for most species, WBCT's in teleosts range below 40 sec. Results with mullet showed greatly increased clotting time (more than 60 sec) in 93 percent of the fish. In most of these, no total clotting occurred and even incipient clotting was frequently absent. Observation of fresh samples by phase contrast microscopy showed little or no fibril formation, poor erythrocyte adherence, and no evidence of clot retraction. However, in a few fresh uncoagulated samples, short fibrin fibrils had already formed, presumably prior to withdrawal as is seen in diffuse interstitial coagulation syndrome. Macroscopically, clots when produced remained soft, and no clot retraction or attendant serum exudation occurred.

In the initial cases, hematocrit was measured in centrifuge tubes after the blood had been spun down to separate plasma for chemical analysis. Later, routine microhematocrits were run. The data consistently indicated a strong tendency toward increased hematocrits in fishes exposed to red tide. Measured PCV ranged from 60 to 75 percent, averaging near 70 percent or approximately twice the normal value (Table 2). Although these deviations from normality are much greater than those commonly encountered in human hematology, it may be significant that a strong positive correlation has been found between hyperviscosity and increased hematocritis in human blood (Evans et al., 1971; Purzanski and Watt, 1972; and Barbee, 1973).

Quantitative measurements of plasma hemoglobin are presently incomplete, but observations indicate patent plasma hemoglobin in most cases, frequently at very high levels (Table 2). Pigmentation was commonly so dense that it was difficult to visually differentiate the plasma fraction from the cell pellet in centrifuged 10 ml tubes.

Plasma debris was obvious in 88 percent of the fish when fresh whole blood was examined by phase microscopy (Table 2). Debris in most cases consisted of minute, dark particles of various sizes and shapes accompained by minute spherical, refractile, lipid-like droplets. Free nuclei similar to those of erythrocytic cells were sometimes present as were blood corpuscle ghosts. Patent plasma hemoglobin and plasma debris are suggestive of erythrocyte hemolysis. The severity seems to be generally much greater than hemolysis resulting from stress. A few fishes showed post-mortem bacteremia and were not considered in this paper.

Hemacytometer counts of erythrocytes, normoblasts (achromic pronormoblasts or erythroblasts), thrombocytes, and leucocytes excepting thrombocytes were made (Table 3). Differential counts on stained smears will be made later to increase the accuracy of

the thrombocyte and leucocyte counts and to quantify the remaining types of leucocytes. Preliminary hemacytometer counts, however, showed considerable consistency and reproducibility in some species such as mullet and ladyfish, and an equal amount of variability in others. Some in this latter group showed marked differences between findings by phase contrast microscopy of fresh, whole blood wet mounts and by hemacytometer counts of diluted blood (Table 4). Seatrout and pinfish data was especially variable, the inconsistencies possibly being procedural artifacts.

Results of preliminary blood counts, when compared to norms for each species, indicated a very high incidence of normoblastosis (achromic), leucocytopenia, and thrombocytopenia in fishes exposed to G. breve red tide (Table 3). Normoblastosis was severe; normoblasts averaged 48.6 percent of the TBC for all fish tested. Maximum normoblastosis occurred in mullet with a TBC of 60.6 percent normoblasts and minimum in ladyfish with only 8.5 percent normoblasts. These levels range from only slightly above normal to almost 10X the normal level, averaging near 7X the normal level! These high levels may have partially resulted from a response of hematopoetic tissues to stress and hemolysis. Thrombocyte levels were rather severely depressed averaging only 0.31 percent TBC or 55,360/mm^3. This ranged from the extremely depressed average of 0.0 in mullet to a near normal average of 1.36 percent TBC or 96,640/mm^3 in ladyfish. A similar pattern of reduction in leucocytes was seen with an overall average of 0.40 percent TBC, 28,960/mm^3; 0.0 in mullet; and 1.75 percent, 64,640/mm^3 in ladyfish. Counts of normochromic erythrocytes, on the other hand, showed only mild anemia with an overall average of 3,698,000/mm^3 and the average mullet value of 3,069,000/mm^3. Ladyfish showed a normal value of 5,055,000/mm^3. The result of the generally severe normoblastosis but mild normochromic anemia is a markedly increased TBC, averaging 6,343,000/mm^3, and 6,914,000/mm^3 in mullet, a phenomenon sometimes seen in dehydration. These increases could be expected to account for only about one third to one half of the increased PCV if mean cell volume (MCV) had remained constant. It is, therefore, assumed that MCV did not remain constant but increased tremendously to account for the remainder of the PCV increase observed.

Anatomy

Empty stomachs were observed in almost every fish examined. In addition, little or no chyme was present in the intestine of most specimens, including mullet, thus indicating at least 12 to 24 hours fasting prior to death. Although empty stomachs could have resulted from premortal regurgitation, this is unlikely in view of the lack of chyme and the failure to observe regurgitation in distressed specimens.

Overt splenomegally was present in 78 percent of the specimens and in 83 percent of the mullet (Table 2). Although norms are not well known, spleen weights in cases recorded as splenomegally appear to be 175 to 300 percent of normal. The spleen, normally elongate, flaccid, and angular, typically became rounded and turgid when hypertrophic. Color ranged from normal to slightly mottled. Other internal organs did not show any consistent manifest abnormalities. Brain tissue appeared normal to slightly pinkish, a frequent sign of cerebrovascular congestion. Cerebral autolysis seemed to occur with unusual rapidity in those few specimens observed at the longer post-mortem periods. In most of these, bacteremia and other signs of sepsis and putrifaction were present, and data from these fish were not used in any other analyses.

Although few tissues have been histologically processed, initial results show the presence of considerable endothelial hepatic hemosiderin. Most or all marine and fresh water fishes of certain families, such as Mugilidae or Serranidae, typically show some hemosiderin in the internal organs, especially the endothelium of the spleen, kidney, and liver. However, in fishes exposed to red tide, the amount is considerably greater than usual.

Ectoparasites did not seem to be affected by red tide. The usual living copepods were found on skin, fins, and gills along with various gill trematodes, protozoans, and even the dinoflagellate, Oodinium. Some affected parasites could, of course, have dropped off, but if they had, they were not obviously absent and no attachment scars were noted.

Aerosol effects

When G. breve red tides enter the surf zone, an aersol is sometimes generated that may be wafted inland by onshore winds (Woodcock, 1948, 1955; Ingle, 1954; Music et al., 1973). These odorless aerosols frequently cause considerable human discomfort and even debilitation of sensitive individuals. In the first few weeks of the 1974 recurrence of red tide, westerly winds caused locally severe aerosols along the Gulf beaches. During this period, signs of oxygen distress in domestic goldfish (Carassius auratus) were reported by several beach residents. Distress signs were present for several days and about half the affected fish died. All ponds with affected fish were open to the atmosphere and were less than 100 m from red tide affected gulf or bay waters. Some of the affected fish were almost ten years old, and one of the ponds had contained healthy goldfish continuously for 20 years. Two distressed goldfish were collected and subjected to complete necropsy. One died in transit to the laboratory, and the other was sacrificed. Both fish showed signs

congruent with those found in fishes naturally exposed to red tide. Goldfish may be particularly sensitive to the red tide toxin and may thus have been debilitated and killed by toxins carried aerially from the surf into the fish pools. Such an effect on goldfish would be an indication that red tide aerosols, of unknown composition, do contain biotoxins or other substances causative of hemopathy and other signs.

Avian Pathobiology

Sign complexes

Signs of distress among lesser scaup ducks were remarkably uniform. In the early stages, they showed a slight weakness and reluctance to flee when approached. Diseased ducks remained on the shore or water long after unaffected ducks had fled. When affected birds did take flight, they resettled quickly. Weakness progressed until flight became impossible and floating ducks resorted to diving for escape. Checks of normal ducks showed dives to average 31 sec, but sick ones usually remained submerged less than 10 sec, and some could not dive at all. Many of the more seriously ill birds floated very low in the water, often with only the neck and head emergent, due to possible oil gland dysfunction. Many came ashore where they sought out cover in which to hide and ultimately die. Agonal ducks would sit quietly on the beach, often with the head slumped, and made no attempt to flee or defend themselves when approached and picked up.

In the laboratory, prostrate ducks showed a profuse, clear nasal discharge and a translucent, mucoid, oral discharge. A chalky yellow diarrhea was present. Irritability slowly disappeared until no response was elicited, even by venipuncture or cardiac puncture. The conjunctival reflex disappeared although the eyes remained open and clear. The righting reflex was the last to disappear, usually within ten minutes of death. The nictitating membranes were typically edematous, the eyes lacrimating, and the pupils bilaterally dilated but reactive to light. Respiration rate was normal but labored, the mouth often being opened upon each inhalation, and accompanied by subcrepitant rales. Respiration rate increased dramatically to 40 to 55/min (3-4X normal) in about half the birds during the last five min before death. Tachycardia frequently was present (150 to 179/min), and blood pressure seemed to be low as indicated by difficulties in accomplishing arteriopuncture of the brachial artery. Body temperature (normally 40 C) typically fell for several hours before death, commonly as low as 30-31 C. This fall either continued or reversed premortal, rising rapidly as high as 44.5 C. The head was laid down about 10 min premortal, and weak, agonal wing

flapping sometimes occurred.

Measured weight loss during sickness (about 10g/hr) plus observed fluid loss suggest severe dehydration was occurring. Ducks collected before their heads dropped could usually be saved (71 percent) by oral administration of fresh water. Substantial recovery was rapid with disease signs disappearing in one to six hrs. Health and strength returned slowly, with 7 to 14 days required before oil gland function and flight capability were normal.

Pathology

Of 52 lesser scaup examined, 24 were subjected to the complete necropsy procedure. Upon necropsy, these 500-680 g animals showed substantial subcutaneous fat indicating the disease ran its course fairly rapidly without prolonged debilitation. Small sand-dwelling gastropod and bivalve mollusks were commonly present in the proventriculus indicating recent feeding. The remainder of the digestive tract contained normal amounts of lightly yellowish chyme and fecal material. The intestines appeared greyish, and mild splenomegally was usual (Figure 3). Pneumonic subpleural hemorrhages seen in some birds (Figure 2) were apparently dissection artifacts and could be prevented by intracardial aspiration of the systemic blood prior to opening the body cavity. Subperitoneal and interstitial thromboses were seen in pancreases of two exceptional birds (Figure 4) and another showed numerous small subcutaneous hemorrhages and thromboses. (Figure 5). The vascular system seemed unusually fragile. Salt glands were hypertrophic and the brains somewhat pinkish, possibly from vascular congestion. Wet mounts of various tissues and fluids were unremarkable. Intestinal and pleural floras seemed normal. Upon bacteriological and virological examination, other tissues, fluids, and organs were aseptic (Forrester, unpublished). Serological tests for common avian diseases, bioassay for botulism, and anticholinesterase inhibition tests for organophosphate pesticides and other toxins, all run by other laboratories, were negative (Jazman and Stoddard, pers. comm.).

Exactly 60 percent of both the affected and normal appearing ducks showed an overt red tide exposure syndrome as defined from the fish hematological studies. Cyanemia, normoblastosis, increased hematocrit, and increased WBCT were always present (or absent) together. In addition, plasma hemoglobin, serum debris, hyperviscosity, thrombocytopenia, and leucocytopenia were seen in various combinations with the above sign grouping. These signs were much less severe in lesser scaup than in fish, however, running about an order of magnitude less in intensity than in mullet and other fishes. We conclude from this that the

red tide was having a minor affect on a proportion of these ducks, but no correlation existed between the occurrence of red tide sign complex and the lethal syndrome.

Morbidity

Numerous counts of dead and living ducks were made by ornithologists during the die-off. Of 965 sick and dead ducks enumerated, over 80 percent were males. Counts of 3,571 normally-acting ducks on the water showed the sex ratio to be almost even (slightly weighted toward females). Disease incidence in males was thus over four times that of females.

The heaviest kill occurred during the last three weeks of March in Hillsborough Bay where little or no red tide penetrated, but where the largest flocks seemed to have congregated. At least 12,000 and possibly over 20,000 lesser scaup died during the eight week mortality.

Experimental intoxication

Attempts were made to intentionally intoxicate lesser scaup with G. breve red tide in order to compare the resulting sign complex with that observed in the lethal syndrome. After considerable effort, lesser scaup were trapped alive in the St. Marks Wildlife Refuge in north Florida by the Florida Game and Fresh Water Fish Commission. These collections were made outside the Florida west coast area in an attempt to obtain specimens without a recent red tide history. Difficulties were encountered in acclimating these animals to captivity and several died and were subjected to complete necropsy as pre-experimental controls. They seemed to be emaciated and in poorer health than ducks from the red tide area. Toxic clams (Mercenaria campe chiensis) were collected from the red tide area and assayed at 48 mouse units. Water from a concentrated patch of red tide was collected and contained 22 million cells per liter or more than ten times the cell counts in the areas frequented by the ducks. Both clams and water were stored at -25 C. Exposures were conducted at the University of Florida by Dr. Donald Forrester. Ducks were given a 50:50 tapwater:red tide water mix for drinking purposes for 11 days. The following eight days, the red tide drinking water was continued plus each duck was given 20 g of toxic clams per day. They took the clams and water eagerly and at the end of the two week period showed no abnormalities and even an increased vigor. Plans to feed toxic clams to satiation were aborted due to self-inflicted injuries from the birds' escape attempts and due to technical problems. Even though terminated prematurely, this test showed that a total toxin exposure several times the expected

maximum natural exposure produced no manifest disease in these ducks, much less the lethal syndrome observed. This lethal disease thus remains idiopathic.

CONCLUSIONS

The results herein present evidence of severe hematological degradation by the 1973-74 red tide in fishes and, to a lesser degree, in lesser scaup ducks. This red tide was not unusual, being described as moderate in both longevity and intensity. Other parameters such as season, location, movement, species killed, etc., were normal for Florida G. breve red tides. There thus seems to be no reason that the hematological and histopathological data obtained should not be representative.

Although no attempt was made to prove that loss of oxygen-carrying capacity, hyperviscosity, or other ichthyopathologies were the cause(s) of piscine death, the extreme magnitude of these debilitory changes makes them most suspect. The normoblastosis, for example, was at or above the level seen in laboratory cases of chronic fatal branchial hemorrhage.

Synthesis of these data suggested two different, but not exclusive, mechanisms of death. Neurointoxication has been well documented in G. breve red tides and in laboratory work. The sign complex and pathology of ladyfish, bay anchovy, clownfish, and possibly others seemed to be consistent with this etiology. On the other hand, catfish, sheepshead, goldfish, mullet, and others seemed to be consistent with a fatal hemopathy. Seatrout and possibly others may succumb to a combination of the two. A strong interspecific variability in resistance to terminal red tide intoxication was strongly supported.

Although a sign complex suggestive of mild G. breve intoxication was found in some of the lesser scaup, no evidence was found causatively incriminating red tide in these bird deaths. The lethal syndrome thus remains idiopathic.

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LITERATURE CITED

Barbee, J. H. 1973. The effect of temperature on the relative viscosity of human blood. Biorheology 10:1-5.

Blogoslawski, Walter. Pers. Comm. National Marine Fisheries Service. 212 Rogers Ave. Milford, Connecticut 06460.

Brunori, M., B. Giardina, E. Chiancone, C. Spagnuolo, Binotti, and E. Antonini. 1973. Studies on the properties of fish hemoglobins: molecular properties and interaction with third components of the isolated hemoglobins from trout (Salmo irideus). Eur. J. Biochem. 39:563-70.

Cummins, J. M., and A. A. Stevens. 1970. Investigations on Gymnodinium breve toxins in shellfish. Pub. Health Serv. Bull. U.S. Dept. of Health, Educ., and Welfare, Washington, D. C.

Doig, M. T., and D. F. Martin. 1973. Anticoagulant properties of a red tide toxin. Toxicon 11:351-5.

Evans, R. L., R. B. Kirkwood, and D. G. Opsahl. 1971. The dynamic viscosity of some human blood. Biorheology 8:125-8.

Forrester, Donald J. Pers. Comm. School of Veterinary Sciences. University of Florida, Gainesville, Fla. 32601.

Galtsoff, Paul S. 1948. Red tide. Progress report on the investigations of the cause of the mortality of fish along the west coast of Florida conducted by the U.S. Fish and Wildlife Serv., Spec. Sci. Rept. No. 46:1-44.

Glazier, W. C. W. 1882. On the destruction of fish by polluted water in the Gulf of Mexico. Proc. U.S. Nat. Mus. 4:126-7.

Glennan, A. H. 1887. Fish killed by poisonous water. Bull. U.S. Fish Comm. Vol. 6 (1886):10-11.

Heath, Ralph, and Harold Albers. Pers. Comm. Suncoast Seabird Sanctuary, 18323 Sunset Blvd. Redington Shores, Florida 33708.

Hemmert, Wynn. Pers. Comm. Fla. Dept. Health Rehabil. Serv., Div. of Health. 1217 Pearl St. Jacksonville, Fla. 32201.

Ingle, R. M. 1954. Irritant gases associated with red tide. Univ. Miami Mar. Lab., Spec. Serv. Bull. 9:1-4.

Jazman, and Stoddard. 1974. Pers. Comm. Fla. Dept. of Agr. & Consumer Serv. Bureau of Diagnostic Laboratories. Kissimmee, Fla. 32741.

Kim, Y. S., J. R. Linton, and D. F. Martin. 1974. Comparison of rabbit and mullet red cell hemolysis induced by Gymnodinium breve toxin. Toxicon (In Press).

Kim, Y. S., and D. F. Martin. 1974. Effects of salinity on synthesis of DNA, acidic polysaccharide, and ichthyotoxin in Gymnodinium breve. Phytochemistry. 13:533-8.

Kocan, R. M. 1972. Some physiologic blood valves of wild diving ducks. J. Wild. Dis. 8:115-8.

Kocan, Richard. Pers. Comm. Bur. Sportfishery & Wildlife, Patuxet Wildlife Research Center. Laurel, Maryland 20810.

Lucas, A. M., and C. Jamroz. 1961. Atlas of avian hematology. Agric. Monograph 25. U. S. Dept. of Agricul., Wash., D. C.

Magath, T. B., and G. M. Higgins. 1934. The blood of the normal duck. Folia Haematol (Leipz) 51:230-2.

Martin, D. F., and G. M. Padilla. 1971. Hemolysis induced by Prymnesium parvum toxin, kinetics and binding. Biochim. Biophys. Acta 241:213.

Martin, D. F., G. M. Padilla, M. G. Heyl, and P. A. Brown. 1972. Effect of Gymnodinium breve toxin on hemolysis induced by Prymnesium parvum toxin. Toxicon 10:285.

Martin, D. F., and G. M. Padilla. 1973a. Effect of Gymnodinium breve toxin on potassium fluxes of erythrocytes. Toxicon (In press).

Martin, D. F., and G. M. Padilla (eds.) 1973b. Marine pharmacognosy; action of marine biotoxins at the cellular level. Academic Press, New York. 317 p.

McFarren, E. F., H. Tanabe, F. J. Silva, W. B. Wilson, J. E. Campbell, and K. H. Lewis. 1965. The occurrence of a ciguatera-like poison in oysters, clams and Gymnodinium breve cultures. Toxicon 3:111-23

Moe, Martin A. Pers. Comm. AquaLife Research Laboratory, 1000 3rd St. S., St. Petersburg, Fla. 33701.

Moore, M. A. 1882. Fish mortality in the Gulf of Mexico. Proc. U.S. Nat. Mus. 4:125-6.

Music, S. I., J. T. Howell, and C. L. Brumback. 1973. Red tide, its public health implications. J. Florida Mar. Sci. 60(11):27-9.

Overstreet, Robin M. Pers. Comm. Gulf Coast Research Laboratory, P.O. Drawer AG, Ocean Springs, Miss. 39564.

Paster, Z., and B. C. Abbott. 1969. Hemolysis of rabbit erythrocytes by Gymnodinium breve toxin. Toxicon 7:245.

Purzanski, W., and J. G. Watt. 1972. Serum viscosity and hyperviscosity syndrome in IgG multiple myeloma. Ann. Intern. Med. 77:853-60.

Ray, S. M., and W. B. Wilson. 1957. Effects of unialgal and bacteria-free cultures of Gymnodinium brevis on fish, and notes on related studies with bacteria. U.S. Fish Wildl. Serv., Fish. Bull. 123:469-96.

Rounsefell, G., and W. Nelson. 1964. Status of the red tide research in 1964. U.S. Fish Wildl. Serv. Tech. Rep. 64(1): 1-92.

Rounsefell, G. A., and W. R. Nelson. 1966. Redtide research summarized to 1964 including an annotated bibliography. U.S. Fish Wildl. Serv. Spec. Sci. Report. Fish. 535.

Saunders, D. C. 1966. Differential blood cell counts of 121 species of marine fishes of Puerto Rico. Trans. Am. Microscop. Soc. 85(3):427-49.

Sievers, A. 1969. Comparative toxicity of Gonyaylax monilata and Gymnodinium breve to annelids, crustaceans, mollusks and a fish. J. Protozool. 16:401-4.

Sinderman, Carl J. 1961. Serological studies of redfish. Fish Bull. U.S. Fish and Wildl. Serv. 61.

Sinderman, Carl J. 1967. Blood types in fish. Am. Biol. Teach. 29:439-41.

Smith, Gregory B. Pers. Comm. Marine Research Laboratory, Dept. Nat. Res., 100 8th Ave., S. E., St. Petersburg, Fla.

Spiegelstein, M. Y., Z. Paster, and B. C. Abbott. 1973. Purification and biological activity of of Gymnodinium breve toxins. Toxicon 11:85.

Starr, T. J. 1958. Notes on a toxin form Gymnodinium breve. Tex. Rep. Bio. Med. 16:500-7.

Steidinger, K. A. 1973. Phytoplankton ecology: a conceptual review based on the eastern Gulf of Mexico research. CRC Crit. Rev. Microbiol. 3(1):49-68.

Steidinger, K. A., and R. M. Ingle. 1972. Observations on the 1971 red tide in Tampa Bay, Florida. Environ. Lett. 3(4):271-8.

Steidinger, K. A., and E. A. Joyce, Jr. 1973. Florida red tides. Fla. Dep. Nat. Resource, Mar. Res. Lab. Educ. Series, No. 17.

Steidinger, K. A., M. A. Burklew, and R. M. Ingle. 1973. The effects of Gymnodinium breve toxin on estuarine animals, pp. 179-202. In Marine pharmacognosy; action of marine biotoxins at the cellular level. D. F. Martin and G. M. Padilla (eds). Academic Press. New York.

Tester, Lana S. Pers. Comm. Marine Research Laboratory, Fla. Dept. Nat. Res., 100 8th Ave., S.E., St. Petersburg, Fla. 33701.

Trieff, N. M., M. Meshan, D. Grajcer, and M. Alam. 1973. Biological assay of Gymnodinium breve toxin using brine shrimp. Tex. Rept. Biol. Med. 31(3):409-22.

Ward, J. W. 1969. Hematological studies on the Australian lungfish, Neoceratodus forsteri. Copeia 3:633-5.

Ward, J. W., and H. C. Davis. 1974. Ecological and hematological studies on Clarias batrachus (Linne). Univ. of S. Florida. (unpublished).

Ward, W., and G. H. Ezell. 1967. Further blood studies in marine fish. Anat. Rec. 157(2):337-8.

Wilson, W. B., and A. Collier. 1955. Preliminary notes on the culturing of Gymnodinium breve Davis. Science 121(3145):394-5.

Woodcock, A. H. 1948. Note concerning human respiratory irritation associated with high concentrations of plankton and mass mortality of marine organisms. J. Mar. Res. 7:56-62.

Woodcock, A. H. 1955. Bursting bubbles and air pollution. Sewage and Industrial Wastes 27:1189-92.

Table 1. Tissue, sections, and stains for histological preparations

Tissue	Section Orientation	Usual Stains	Electron Microscopy (Sample Location)
Blood	smear	Wrights Giemsa P/S B/H and/or M/G	fixed whole blood
Gills	oblique to filaments perpendicular to plane of gill	B/H and/or M/G H & E	mid-lateral piece from second left arch
Liver	transverse section through gall bladder and one lobe	H & E B/H and/or M/G PAS Perls Fe P/S	posterior tip of left lobe
Spleen	longitudinal section (occasional transverse section when entire viscera sectioned)	H & E PAS Perls Fe P/S B/H and/or M/G	posterior tip, distal to vascular bundle
Gonad	transverse section 2/3 distance from gonoduct (usually only 1 lobe sectioned)	H & E B/H and/or M/G	anterior tip, left lobe
Stomach	longitudinal section running through pyloris, fundus, and esophagus	H & E B/H and/or M/G	none taken

Table 1. (cont.)

Tissue	Section Orientation	Usual Stains	Electron Microscopy (Sample Location)
Intestine	transverse section through intestinal mass (2 sectioning planes often used, one anterior and one posterior)	H & E B/H and/or M/G	illial portion
Heart	saggital section through atrium, ventricle, and conus arteriosus	H & E B/H and/or M/G Perls Fe P/S	lateral edge of ventricle
Kidney, posterior	transverse section, medial	H & E PAS B/H and/or M/G Perls Fe P/S	posterior transverse section or left lateral posterior portion
Kidney, anterior	transverse section, medial	H & E PAS B/H and/or M/G Perls Fe P/S	transverse or lateral edge of left lobe
Brain	saggital section just to left of interhemispheric fissure	H & E PAS B/H and/or M/G S/M A/T	piece from left optic lobe

Table 1. (cont.)

Tissue	Section Orientation	Usual Stains	Electron Microscopy (Sample Location)
Lung (left)	subfrontal section parallel to major plane of pleurus	H & E Giemsa	mediolateral edge
Musculature	transverse section	H & E B/H and/or M/G	mid-dorsal piece from left side
Salt gland (left)	median saggital	H & E	dorsomedial portion

Abbreviations of Stains:

H & E	- Hematoxylin and eosin
B/H	- Brown-Hopps method for gram positive and gram negative bacteria
M/G	- MacCallum-Goodpasture method for gram positive and gram negative bacteria
PAS	- Periodic acid-schiff reaction
P/S	- Puchtler-Sweat method for hemoglobin and hemosiderin
Perls Fe	- Perls' method for iron
S/M	- Sevier-Munger method for neural tissue
A/T	- Aldehyde-Thionin, PAS method for central nervous system

Table 2. Ichthyopathology

	All Fish	Percent	Mullet	Percent
Cyanemia	30	66.67	16	84.21
No cyanemia	13	28.89	3	15.79
No data	2	4.44	0	0
Increased viscosity	18	40.00	11	57.89
Nomal viscosity	17	37.78	2	10.53
Decreased viscosity	3	6.67	1	5.26
No data	7	15.55	5	26.32
Plasma debris	23	51.11	9	47.37
No debris	3	6.67	3	15.79
No data	19	42.22	7	36.84
Clotting time				
0-15 seconds	8	17.78	0	0
15-45 seconds	7	15.56	1	5.26
46-150 seconds	4	8.89	0	0
151-00 seconds	20	44.44	14	73.68
No data	6	13.33	4	21.05
Splenomegaly	25	47.17	9	47.37
No splenomegaly	7	13.21	2	10.53
No data	21	39.62	8	42.10

Table 3. Ichthyohematology

	Erythro-cytes	Normo-blasts	Leuco-cytes	Thrombo-cytes	Total
All counts (30)	3,698,320	2,603,120	28,960	55,360	6,342,720
Mullet (15)	3,069,200	3,823,440	640	24,080	6,913,600
Ladyfish (6)	5,055,280	436,800	64,640	96,640	5,653,440

Table 4. Hematological technique comparison of average percentage composition for wet mount and hemacytometer

Total				
wet mount (29)	67.96	31.45	.52	.68
Computed (26)	50.62	48.60	.40	.31
Mullet				
wet mount (13)	50.69	49.15	.46	.38
computed (15)	39.35	60.65	.00	.00
Ladyfish				
wet mount (6)	94.66	5.00	.517	.217
computed (6)	88.28	8.51	1.75	1.36

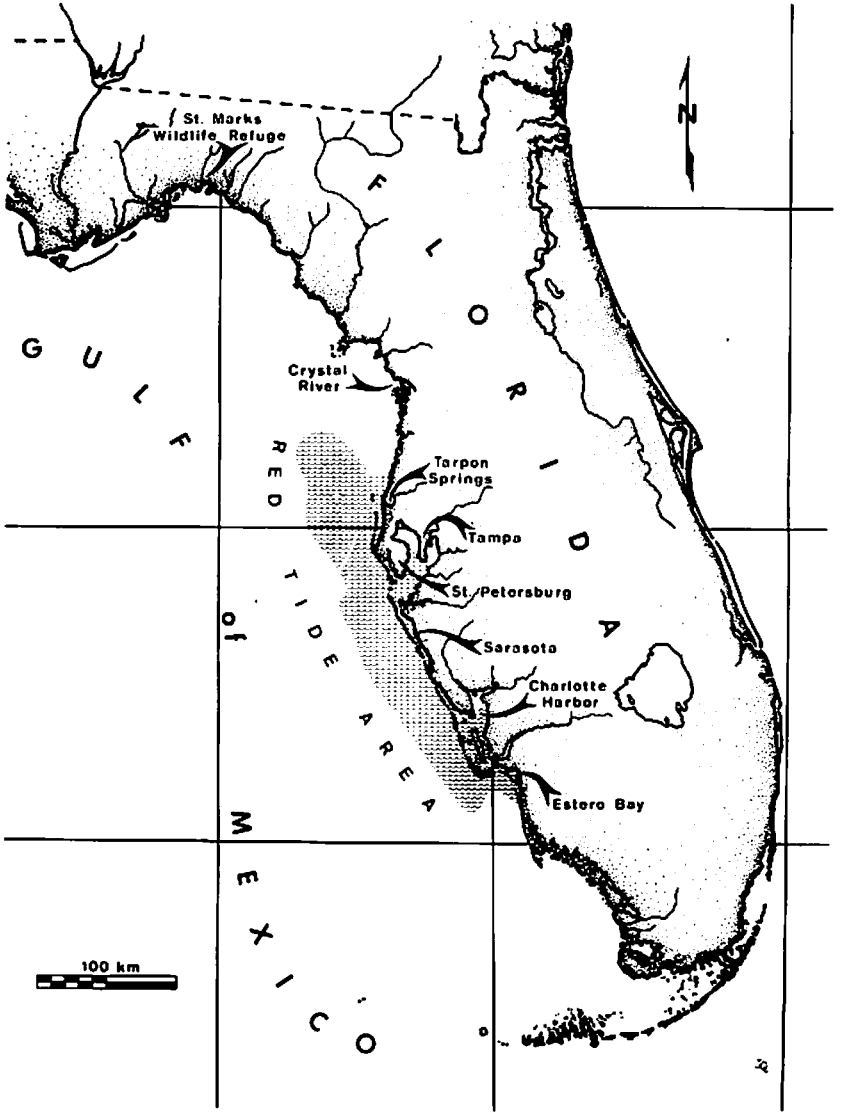

Figure 1. Map showing area of 1973-1974 Gymnodinium breve red tide and collection points for control specimens. The seaward limit shown is an estimate based on the limited number of samples taken far offshore.

Pathologies of the Lesser Scaup,
Aythya affinis

Figure 2. Ventral view of lungs *in situ* against the rib cage showing generalized hemorrhage and resultant thromboses with partially atelectatic right pulmo. This pathology seems to be a dissection artifact, possibly related to excessive vascular fragility or angiasthenia.

Figure 3. Abdominal organs exposed by abdomino-thoracic resection to show pronounced splenomegally. The lungs appear normal in this animal in which an intracardial blood evacuation preceded sternocostalectomy. S, spleen; P, left lung; C, heart; H, liver; V, proventriculus; G, gizzard; I, intestine.

Figure 4. Pancreas with subperitoneal and interstitial thromboses.

Figure 5. Medial side of abdominal integument showing numerous subcutaneous thromboses.

(Scales in centimeters)

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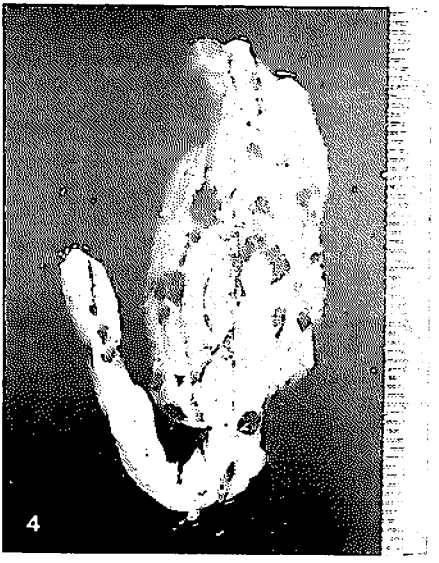

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DISTRIBUTION OF CHITINOCLASTIC BACTERIA IN NATURAL ESTUARINE WATERS AND AQUARIAL SYSTEMS[1]

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INTRODUCTION

The role of chitinoclastic microorganisms as causative agents of certain diseases of aquatic animals is well documented (Sindermann, 1974). In general, chitinoclastic bacteria may be categorized by their ability to produce a diversity of disease conditions. These organisms may be disease initiators; i.e., they may colonize the exoskeleton surfaces of crustaceans and elaborate the necessary hydrolytic enzymes for cuticle breakdown resulting in loci for penetration by opportunistic pathogens. Numerous shell diseases have been reported in which chitinoclastic bacteria such as Beneckea, Vibrio, Pseudomonas, and Aeromonas species were consistently identified with the lesions of shrimp, crab, lobster, and crayfish (Cook and Lofton, 1973; Rosen, 1970). Secondly, chitinoclasts may be directly toxic or possess the intrinsic capacity to induce a disease syndrome by toxin production. For example, V. parahaemolyticus (Vanderzant et al., 1970; Krantz et al., 1969), V. anguillarum (Evelyn, 1971), and V. alginolyticus (Tubiash et al., 1970)

[1] This work is a result of research sponsored by NOAA office of Sea Grant Development, Department of Commerce, under Grant 04-3-158-19.

have been suggested as causative agents of death in shrimp, crab, mollusks, and salmon. Certainly, other examples of host-related activities of chitinoclastic microorganisms could be cited to illustrate the significance of this diverse group in the inception of pathogenic conditions.

The purpose of the present study was to determine the seasonal distribution of chitinoclastic bacteria in estuarine environments and in healthy aquarial systems in order to define some of the ecological parameters which influence chitinolytic populations and their activity. A more extensive review of the biology of these microorganisms is presented elsewhere (Hood and Meyers, 1973). Implications of such data in the inception of specific disease problems will be discussed subsequently.

MATERIALS AND METHODS

Sampling

Collections were made within Airplane Lake, a small semi-enclosed, saline water lake noted for its high productivity of both brown and white shrimp (see L.S.U. Coastal Studies Bulletin No. 5, 1970, for a detailed description of the environment). Three site locations were selected: at the mid-channel entrance, in the middle of the first lobe of the lake, and at the furthermost point back into the second lobe of the lake. Samples of submerged sediment (using a Peterson grab) and sea water (at a depth of one foot from surface) were taken monthly during 1971. Shrimp samples were collected for digestive tract analysis during May, June, August, September, and October, 1971. Exoskeleton analyses were conducted using shrimp collected monthly the following year. Shrimp (approximately 10 cm in length) were taken from the trawl by hand, placed in sterile plastic bags, and returned to the laboratory in an ice chest (approximately 4-6 C) for examination within four to six hours after collection. Analysis also was performed at the dock within five minutes after collection. Bacterial populations were comparable to those found in animals returned to the laboratory for study.

Shrimp for aquarial tests were collected, maintained in aerated plastic containers at approximately 20 C, and returned to the laboratory within 24 hours. The animals were then transferred to 50 gallon glass tanks, containing natural sea water, which had been set up one week prior to receiving the shrimp. The tanks contained a washed (hot water) coarse sand bottom with appropriate aeration pumps and filters and were allowed to aerate and filter five days before transfer of the animals. Water quality was maintained by visual observation; there was no evidence of fouling or

of an increase in total heterotrophic aerobic bacterial populations. Shrimp were maintained for two weeks under these conditions, following which chitinoclastic bacteria were enumerated. Diets consisted of a formulated alginate-bound crustacean diet (Meyers and Zein-Eldin, 1973), added in amounts such that little extraneous food accumulated in the system.

Preparation of Sample for Bacterial enumeration

For bacteriological analysis of the shrimp, a sterile graduated Pasteur pipette was inserted carefully into the proventriculus and the contents removed by suction. The intestinal tract was removed aseptically by bisecting the animal behind the hepatopancreas gland. With a sterile forceps, the intestine was clasped and the tail of the animal pinched, following which the gut was easily removed. The intestines were weighed and placed in tubes of sterile sea water (to give a 1/100 dilution) containing glass beads. The sample was shaken for one minute to rupture the gut lining and to evenly disperse the contents.

The hepatopancreas was removed aseptically by careful dissection, weighed, and placed in tubes of sterile sea water containing glass beads. The water blank was shaken for one minute to evenly disperse the tissue. Shrimp exoskeleton was removed aseptically, weighed, and placed in sterile sea water with homogenization performed in a micro-blender (Lourdes Instrument Corp. Model VM) at 8,000 rpm for two minutes. The blender cup was submerged in an ice bath during the operation to avoid heat damage to the bacteria present.

Enumeration of Chitinoclastic Bacteria

One milliliter of the appropriate dilution of sediment, sea water, and shrimp tissue was placed on the surface of a thin layer of basal sea water agar. Chitin agar medium, containing 0.05 percent yeast extract, 2.5 percent ball-milled (48 hours at 2 C) purified chitin (Calbiochem), and sea water of pH 7.6 cooled to 45 C, was poured over the inoculum. The plates were swirled rapidly to insure mixing and placed on a cooled surface to reduce bacterial heat damage. After ten days of incubation at 22 C, colonies which exhibited clearing zones indicative of chitin utilization were enumerated.

RESULTS AND DISCUSSION

The seasonal distribution of chitinoclastic bacteria in the estuarine environment of Airplane Lake is shown in Figure 1. Optimum populations in the sediments occurred during April, followed by two secondary peaks in June and August. Maximal concentrations of chitin utilizers in the water column occurred in February and March, with secondary peaks in May and June. No direct correlation between numbers of chitinoclastic bacteria and temperature was evident. As noted in Table 1, highest temperatures were in late summer (August) while greatest numbers of chitinoclasts occurred during the spring and early summer. Optimum populations of chitinoclastic bacteria, however, occurred when median temperatures were above 16.9 C. Data from other workers (Loesch, 1971; Thomas, 1974) have shown that chitin-producing animals reach peak densities in spring and early summer, i.e., copepods in April, amphipods in February and March, larvae and juvenile brown shrimp in April and May, and larvae and juvenile white shrimp in June and July. It is entirely feasible that the chitinoclastic bacterial population and its ultimate biomass may be dependent primarily on chitinous substrate input into the estuarine system.

Buck and Barbaree (1971) detected higher concentrations of chitinoclastic bacteria attached to copepods than in surrounding waters, suggesting the presence of an indigenous or closely associated bacterial flora. Seki and Taga (1963) observed a similar trend in oceanic waters off the coast of Japan. These observations are supported by data presented in Table 2, which gives the average number of chitin-utilizing bacteria associated with healthy white shrimp from Airplane Lake and with the surrounding waters from which they were collected and the average number of such bacteria associated with waters and shrimp under normal or healthy aquarial conditions. It appears that aquatic animals such as shrimp both in natural waters and in aquaria have a certain "background level" of associated chitinoclastic bacteria. It is interesting to note that the concentrations of bacteria in waters and those attached to the shrimp under healthy aquarial conditions were similar to those of natural waters. Figure 2 presents the number of chitinoclasts associated with the exoskeleton and digestive tract of white shrimp during the warmer months. The greatest external population was evident during the month of August, suggesting a temperature-related phenomena. In contrast, the internal bacterial population remained relatively constant.

It is generally recognized that the total bacterial biomass of a particular physiological group does not always reflect the activity of that group. In an effort to obtain an additional measurement of the rate of chitin decomposition, substrate was placed *in situ* and weight loss was measured. These data will be reported

elsewhere but can be summarized briefly (see also Chan, 1970). The rate of chitin degradation is directly related to initial substrate concentration, certain organics, particle size, and temperature. The maximum population of chitinoclastic bacteria (colonization) was on sterilized chitin substrate eight days after *in situ* seeding when water temperatures ranged from 27-30 C.

SUMMARY

Little control of chitinoclastic bacteria is possible in natural environments; thus disease conditions incited by these organisms can hardly be controlled in such natural systems. However, under aquarial or maricultural conditions, data from other workers as well as that obtained in our investigations may be applied to alleviate disease problems caused by chitinoclastic bacteria. The following approaches can be suggested:

Since chitinoclastic populations are substrate related, molted exoskeleton should be removed from the culture system as soon as possible to prevent increases in populations of chitinoclastic bacteria. This has particular application in aquaria and in high density stocking situations where exoskeletons can be readily removed.

Molted exoskeleton should be removed from the culture system as soon as possible to prevent increases in populations of chitinoclastic bacteria. This has particular application in aquaria and in high density stocking situations where exoskeletons can be readily removed.

Care should be taken to minimize bruising, handling, and overcrowding. Breakage of the exoskeleton allows increased surface area for colonization by chitinoclasts. In addition, excessive handling or overcrowding may adversely stress the animal making conditions more favorable for proliferation of opportunistic pathogens.

Levels of organics (other than chitinous material) should be kept as low as possible in the immediate environment of the animal. Overfeeding or use of diets with poor water stability will directly affect the organic load in the system, leading to increases in the total bacterial biomass. The latter include not only chitinoclasts but also specific physiological types which can deleteriously affect the animals directly or indirectly by causing serious imbalances in the culture system.

Selection of a "compromise" temperature must be made for good growth of the animal but not at the risk of optimal bacterial growth. This is becoming more of a realistic consideration in

view of the attention being given to use of thermal waters for cultivation of a variety of crustacean species. Since marine chitinoclastic bacteria generally show maximum growth (and chitinase activity) at 27-30 C, selection of a lower temperature may prevent or retard some of the disease problems associated with these bacteria.

ACKNOWLEDGEMENT

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LITERATURE CITED

Buck, S.K., and J.M. Barbaree: 1971. Chitinolytic bacteria associated with marine Copepods. Bact. Proc. 71:G148.

Chan, J. C. 1970. The occurrence, taxonomy and activity of chitinoclastic bacteria from sediment, water, and fauna of Puget Sound. Ph.D. Dissertation, U. of Wash., Seattle.

Cook, D. W, and S. R. Lofton. 1973. Chitinoclastic bacteria associated with shell disease in Penaeus shrimp and the blue crab (Callinectes sapidus). J. Wildl. Dis. 9:154-9.

Evelyn, T. 1971. First records of vibriosis in Pacific salmon cultured in Canada, and taxonomic status of the responsible bacterium, Vibrio anguillarum. J. Fish. Res. Bd. Can. 28:517-25.

Hood, M. A., and S. P. Meyers. 1973. The biology of aquatic chitinoclastic bacteria and their chitinolytic activities. La mer (Bulletin de la Societe franco-japonaise d'oceanographie) 11: 213-29.

Krantz, G. E., R. R. Colwell, and E. Lovelace. 1969. Vibrio parahaemolyticus from the blue crab Callinectes sapidus in Chesapeake Bay. Science 164:1286-7.

Loesch, H. 1971. Some observations on amount of nannoplankton, nematodes, copepods, fish and shrimp found in the Barataria Bay area, Louisiana. L.S.U. Coast. Stud. Bull. No. 6:38-44.

Meyers, S. P., and Z. Zein-Eldin. 1973. Binders and pellet stability in development of crustacean diets. Proc. Third Annual Workshop, World Mariculture Society, 351-64.

Rosen, B. 1970. Shell disease of aquatic crustaceans. In Symposium on Disease of Fishes and Shellfishes (S. Snieszko, ed.). Spec. Pub. No. 5, Amer. Fish. Soc., Washington D.C. pp. 409-15.

Seki, H., and Taga. 1963. Microbiological studies on the decomposition of chitin in marine environments. III. Aerobic decomposition by the isolated chitinoclastic bacteria. J. Ocean. Soc. Jap. 19:143-51.

Sindermann, C. 1974. Handbook of Diagnosis and Control of Diseases in Mariculture. Informal Rept. No. 19, Middle Atlantic Coastal Fish. Center, Oxford, Maryland.

Thomas, J. 1974. Benthos. In Environmental Assessment of A Louisiana Offshore Oil Port and Appertinent Storage and Pipiline Facilities. Technical Appendix VI. 6, Volume II. Center for Wetland Resources, Louisiana State University, Baton Rouge, La. (In press).

Tubiash, H. S., R. R. Colwell, and R. Sakazuki. 1970. Marine vibrios associated with bacillary necrosis, a disease of larval and juvenile bivalve mollusks. J. Bacteriol. 103:272-3.

Vanderzant, C., R. Nickelson, and J. Parker. 1970. Isolation of Vibrio parahaemolyticus from Gulf Coast shrimp. J. Milk Food Technol. 33:161-2.

Table 1. Median temperatures of the surface waters in the Barataria Bay estuary*

Month (1971)	Median Temperature, C
January	14.3
February	16.9
March	17.0
April	22.0
May	25.4
June	26.5
July	27.2
August	29.0**
September	26.4
October	24.0
November	18.2
AVERAGE MEDIAN	20.6

* Data taken at Grand Terre, Louisiana, and provided by the Louisiana Department of Wildlife and Fisheries.
** Highest median temperature.

Table 2. Biomass of chitinoclasts associated with Penaeids

Natural environment	No./g or No./ml
Digestive Tract	
Stomach	1.3×10^7
Gut (mid-hind)	8.0×10^7
Hepatopancreas gland	5.0×10^4
Intact exoskeleton of shrimp from environment	5.0×10^5
Surrounding waters	4.1×10^3
Aquarial Environment	
Intact exoskeleton of shrimp from aquaria	1.5×10^5
Molted exoskeleton from aquaria	6.3×10^7
Surrounding aquarial waters	1.1×10^3

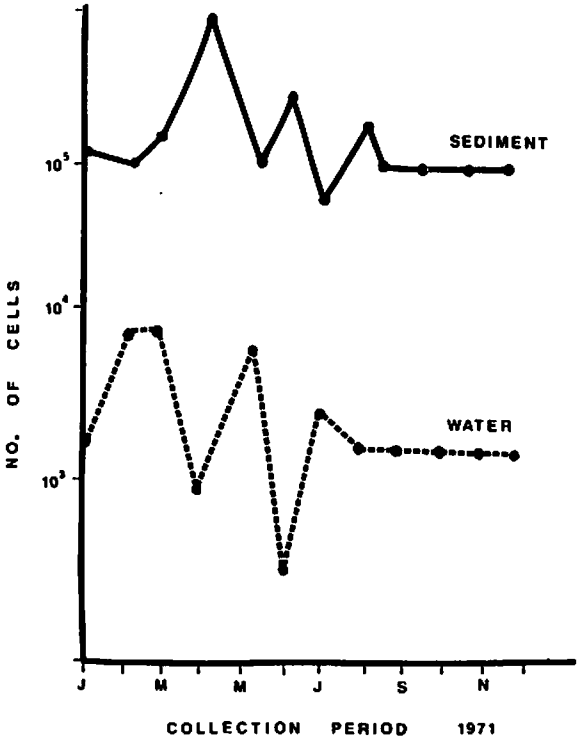

Figure 1. Monthly average for chitinoclastic biomass for sediments and waters in Airplane Lake

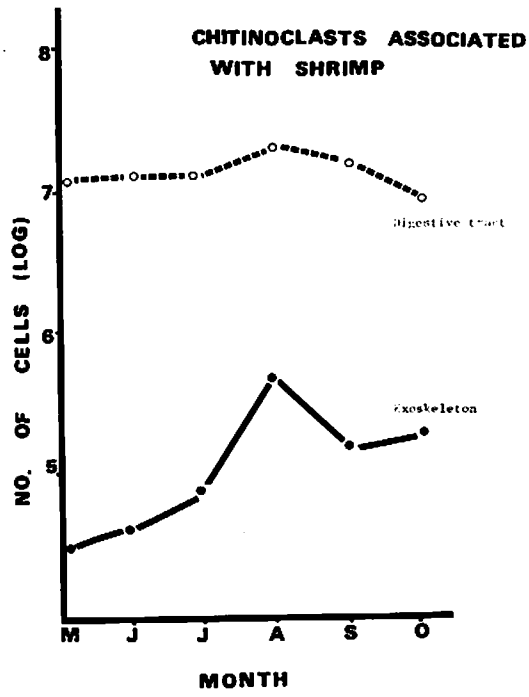

Figure 2. Number of chitinoclastic bacteria per g (wet wt) associated with white shrimp collected from Airplane Lake (1972)

PARASITES OF POMPANO, *Trachinotus carolinus* (LINNAEUS) AND STRIPED MULLET, *Mugil cephalus* LINNAEUS BEFORE AND AFTER CAGE CULTURE[1]

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ABSTRACT

A sample of 20 specimens each of pompano, *Trachinotus carolinus* (Linnaeus), and striped mullet, *Mugil cephalus* Linnaeus, was examined for parasites before (preculture) and after (post-culture) stocking in cages used in mariculture experiments at Dauphin Island, Alabama. Post-culture examination included samples given prophylactic treatments with both formalin and Dylox and with formalin only. Additional post-culture samples of ten specimens of pompano from a 0.1 hectare brackish water experimental pond and ten specimens of wild striped mullet were examined for parasites. There was very little variation in incidence and intensity of parasite infestation with groups of fishes treated with formalin and with formalin and Dylox. Pre- and post-culture parasite data from wild striped mullet were very similar. This supports the accuracy of comparisons using pre-culture data. Prophylactic treatment of fishes prior to and after stocking had no significant effect on the incidence or intensity of infestation of external parasites in the post-culture samples. Post-culture pond and cage parasite data

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were very similar; however, data for some parasite types from the pond culture resembled that for the pre-culture wild specimens.

INTRODUCTION

In 1969 a mariculture experiment was conducted by the Marine Resources Division of the Alabama Department of Conservation and Natural Resources at the Alabama Marine Resources Laboratory on Dauphin Island, Alabama, to develop techniques of culturing marine fishes in floating cages (Swingle, 1970). Fishes were examined to determine the abundance of parasites before the mariculture experiment and at its conclusion (Williams, 1972). The mariculture experiment was continued in 1970 (Swingle 1971a, b; Swingle and Tatum, 1971), and the present parasite study reports the results of parasite examinations of pompano[1], Trachinotus carolinus (Linnaeus), and striped mullet, Mugil cephalus Linnaeus, conducted during this period. The objectives of the following experiment were to obtain some indication of the changes in species of parasites and quantity of parasites of fishes held in mariculture conditions, to compare the parasites of wild and cultured striped mullet during the post-culture period, to obtain some idea of the effect of seasonal variation on the accuracy of pre-culture wild fish data, to compare the parasites of cage- and pond-cultured pompano, and to evaluate the success and effect of prophylactic parasite treatments.

MATERIALS AND METHODS

Pompano and striped mullet were collected in May and July 1970 by 4.5 and 15.2 m seines and held in indoor tanks prior to stocking. Pompano averaged 8.4 cm and striped mullet averaged 4.6 cm in total length. Twenty specimens of each species were examined immediately after capture, and represent the pre-culture sample. Then all fish were treated for 3 hours in 100 ppm formalin prior to stocking. The majority of each species was treated for 24 hours with 0.25 ppm Dylox shortly after stocking. Fish receiving only the formalin treatment will be referred to as formalin-treated, fish receiving both formalin and Dylox as Dylox-treated. Twenty specimens, ten Dylox-treated and ten formalin-treated of both species were examined from the cages at the conclusion of the mariculture experiment during November, 1970, and represent the post-culture sample. Dylox-treated pompano averaged 14.4 cm and formalin-treated averaged 16.1 cm in total length. Striped mullet averaged 16.0 and 7.3, respectively.

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[1] Senior author does not agree with the common name assigned by Bailey et al. (1970).

At the conclusion of the mariculture experiment, a sample of 10 wild striped mullet averaging 22.0 cm in total length was examined and represented a wild-check sample. Wild pompano were not available but a post-culture sample of 10 pompano averaging 21.9 cm in total length was examined from a 0.1 hectare renovated brackish water pond and represented a post-culture pond sample. All fishes used in the experiment were fed commercial trout chow.

Examination of all fishes included examination of the body and fin surface, gill filaments, gill and oral cavity, alimentary system, coelomic cavity, and blood. Head sinuses, urinary bladder, and other tissues of many fish were examined. Smears from approximately five cm^2 of the mucosal surface of body and fins, from approximately one cm^2 of gill filaments, and from two drops of fresh blood were examined in wet mounts with a compound microscope. Gill filaments, gill cavity, oral cavity, body and fin surfaces, alimentary system, coelomic cavity, and mesentery were examined under a dissecting microscope. Protozoans were relaxed using the 1:4,000 formalin method of Rogers (1966) and were preserved in five percent formalin. Monogenetic and digenetic trematodes, cestodes, and nematodes were fixed in hot five precent formalin. Acanthocephala were placed in chilled distilled water until relaxed (usually 24 to 48 hours) and preserved in five percent formalin. Copepods and isopods were fixed in five percent formalin, and leeches were flattened between slides and fixed in five percent formalin.

Parasites were removed, counted, and recorded as very light (VL = 1 to 5 parasites), light (L = 6 to 10 parasites), moderate (M = 11 to 50 parasites), heavy (H = 51 to 100 parasites), and very heavy (VH = 100+ parasites). Location of collection and occasionally more specific data were also recorded. Most parasites were reported as taxonomic groupings that may be controlled by similar prophylactic treatments. Protozoa were reported by genera to allow a more complete explanation. More detailed identifications will be reported elsewhere. Trichodina spp., Scyphidia spp. (Protozoa:ciliata) and Trypanosoma spp. (Protozoa:mastigophora), will be referred to as Trichodina, Scyphidia, and Trypanosoma, respectively.

RESULTS

Pompano

The incidence of Trichodina increased from 10 to 60 percent and the maximum intensity of infestation on the skin increased from very light to moderate after confinement in cages (Table 1).

The incidence and maximum intensity of infestation of Trichodina on the skin in the post-culture pond sample was similar to the pre-culture sample. Incidence of Scyphidia on the skin decreased slightly; also the maximum intensity decreased in the post-culture cage samples. In the post-culture pond sample, the incidence of Scyphidia remained the same.

Trichodina incidence and intensity of infestation increased from 10 to 100 percent on the gill filaments in the post-culture cage and pond samples. Incidence of Scyphidia decreased in post-culture samples from the cages and pond. Maximum and modal intensity increased in the post-culture pond sample. The abundance of monogenetic trematodes increased in post-culture cage and pond samples, from zero to an average of 43 percent infestation (Table 1). Isopoda and copepods were not found in either pre-culture or post-culture samples.

Cestodes were present in all specimens of the pre-culture smaple with 70 percent of them heavily infested. No cestodes were recovered from the post-culture cage and pond samples. The incidence of digenetic trematodes decreased in the post-culture samples. Nematodes occurred only in the mesenteries of the post-culture pond sample.

Striped Mullet

The incidence of Trichodina on the skin increased in the post-culture cage samples (Table 2). The incidence in the pre-culture and in the post-culture wild samples were identical. Scyphidia was rare on the skin in the post-culture cage samples and was not observed in the pre-or post-culture wild samples. Copepods rarely occurred on the skin in the pre-culture and post-culture wild samples; they were absent in the post-culture cage samples (Table 2).

The incidence of Trichodina on the gills decreased in the post-culture cage samples. The maximum intensity of infestation decreased in the post-culture cage samples when compared to the pre-culture and post-culture wild samples.

Scyphidia rarely occurred on the gills of the post-culture cage samples and was absent on the pre- and post-culture wild samples. Monogenetic trematodes on the gills decreased in the post-culture cage samples. The incidence and maximum intensity of copepods on the gills decreased in the post-culture cage samples (Table 2).

The incidence of digenetic trematodes increased while the modal intensity decreased in the post-culture cage samples. Nema-

todes rarely occurred in the kidney in the pre-culture samples and did not occur in the post-culture samples. Blood flagellates decreased in incidence and intensity in post-culture samples.

Parasite Types

None of the study specimens were free of parasites. The percentage of pompano with 1 to 3 parasite types (taxonomic groupings as considered previously) increased from 25 to 40 percent and the percentage of fish with 4 or more parasite types decreased from 75 to 60 percent in the post-culture samples (Table 3). Striped mullet with 1 to 3 parasite types increased from 0 to 60 percent and the percentage of fish with 4 or more parasite types decreased from 100 to 40 percent in the post-culture cage samples.

DISCUSSION

Damage to the host by protozoans and monogenetic trematodes has been discussed by Williams (1972). Finucane (1969) found that isopods did not appear to damage pompano. The authors have not observed damage to pompano infested with isopods but have observed erosion of the gill filaments and occasionally the opercular flap of other species infested with isopods of similar size.

The number of parasite types significantly decreased during culture as was observed in the previous experiment reported in Williams (1972). The incidence of Trichodina greatly increased on the gills and skin during culture of pompano. Only the incidence of Trichodina on the gills greatly increased in the previous experiment. The increase in the incidence of monogenetic trematodes was much greater than in the previous experiment. Incidence of Scyphidia on the skin and gills did not show a significant increase in post-culture samples in either experiment.

In the 1969 experiment 75 percent of the pompano samples were infected with digenetic trematodes and 60 were heavily infected. However, no digenetic trematodes occurred in the post-culture samples. Less spectacular reduction of infections of digenetic trematodes occurred in the other mariculture fishes studies. The availability of intermediate hosts was probably reduced by the modified environment of the cage, or the artificial food replaced the intermediate host in the diet. In the present study all pre-culture samples of pompano were infected with immature cestodes, and 70 percent were heavily infected. However, no cestodes occurred in the post-culture samples. No evident pattern occurred in the other mariculture fishes studied as in the digenetic trema-

tode samples. In pompano and sea catfich, cestodes increased respectively 70 and 85 percent in level of infection from the pre- to the post-culture samples In mariculture fishes held in a cage environment and given an artificial diet, both of which factors should have reduced the number of available intermediate hosts, the level of cestode infection varied from being greatly reduced to greatly increased. Either an intermediate host flourished in the cage environment, or the number of pre-culture samples was inadequate to detect a great variation in level of infection by cestodes and the cestodes were retained throughout the mariculture experiment.

The incidence and intensity of parasite types of the post-culture pond sample of pompano were similar to the post-culture cage sample as would be expected because of the similar culture situations. However, some parasite types from the pond sample resembled parasite types of the pre-culture sample in incidence and intensity, probably a reflection of the lower density and more nearly natural conditions of the pond culture.

Trypanosoma occurred in the blood of pompano and striped mullet. No blood flagellates have been previously recorded from pompano or from striped mullet in North America. Their presence is being further studied by the senior author. Scyphidia, also occurring on all fishes examined, represents a new species and is being studied by the authors. A variety of new species, host, and locality records have been discovered in the present study and will be reported elsewhere.

To provide some indication of seasonal variation in parasite types and thus determine the reliability of pre-culture wild fish data, a post-culture sample of wild striped mullet was examined. Wild pompano were not available during post-culture examinations. The post- and pre-culture wild fish parasite data were very similar (Table 2). This supports the use of pre-culture parasite data. Pre-culture wild fish parasite examinations involve the risks of parasite changes due to the age and size of fish and due to seasonal variation. Post-culture wild fish examinations involve the greater risk of population variation and sample availability (for example, samples of pompano were not available for post-culture examinations). Ideally, pre- and post-culture examination should be made of wild fish for the evaluation of changes in parasites of cultured fish.

Prolonged treatment with 15 ppm formalin for 24 hours was found to be ineffective in eliminating Trichodina, Scyphidia, and monogenetic trematodes on pompano and striped mullet (Williams, 1972). The fishes were treated with 100 ppm formalin for 3 hours and some were also treated with 0.25 ppm Dylox for 24 hours. There was very little difference in incidence or intensity of parasite

infestation in groups of fishes treated with formalin and with formalin and Dylox. The formalin treatment was found to be effective in eliminating Scyphidia and Trichodina in pompano tested in aquaria. Levels of parasite infestation after culturing could either be the result of the treatment failing to completely eliminate the parasites or reinfestation from the environment. In the previously mentioned experiment (Williams, 1972), the prophylactic treatment employed has been shown to be totally ineffective in eliminating parasites. In the present experiment the prophylactic treatments employed prior to and shortly after stocking have been shown to be completely effective in eliminating external protozoa. However, the levels of incidence and intensity of infestation of external protozoa at the conclusion of each mariculture experiment were very similar. Prophylactic treatment of fishes prior to and shortly after stocking had no significant effect on the incidence or intensity of infestation of external protozoa found in the post-culture samples.

Acknowledgments

Appreciation is extended to Wayne E. Swingle, Hugh A. Swingle, Honie H. Crance, and other personnel of the Alabama Marine Resources Laboratory for assistance and loan of facilities. Special thanks is extended to Rene F. Sanchez of the Department of Fisheries and Allied Aquacultures of Auburn University for translation of the abstract.

LITERATURE CITED

Bailey, R. M., J. E. Fitch, E. S. Herald, E. A. Lachner, C. C. Lindsey, C. R. Robins, and W. B. Scott. 1970. A list of common and scientific names of fishes from the United States and Canada. Amer. Fish. Soc. Spec. Publ. 6.

Finucane, J. H. 1969. Ecology of the pompano (Trachinotus carolinus) and the permit (T. falcatus) in Florida. Tr. Amer. Fish Soc. 98:478-86.

Rogers, W. A. 1966. Three species of Pseudomurraytrema (Trematoda:Monogenea) from gills of catostomid fishes. J. Parasit. 52:462-5.

Swingle, W. E. 1970. Experiments in the culture of marine species in floating baskets. Alabama Department of Conservation and Natural Resources, Marine Resources Division, Federal Aid Annual Progress Report 2-86-R-1. Mimeograph File Report 26 p.

Swingle, W. E. 1971a. A marine cage design. Prog. Fish-Cult. 33:102.

Swingle, W. E. 1971b. Striped bass, Marone saxotilis, production to establish commercial stocks in Alabama estuaries. Alabama Department of Conservation and Natural Resources, Marine Resources Division, Federal Aid Annual Progress Report AFC-3-1. Mimeograph File Report. 16 p.

Swingle, W. E., and W. M. Tatum. 1971. Experiments in the culture of marine species in floating baskets. Alabama Department of Conservation and Natural Resources, Marine Resources Division, Federal Aid Annual Progress Report 2-86-R-2. Mimeograph File Report. 26 p.

Williams, E. H., Jr. 1972. Parasitic infestation of some marine fishes before and after confinement in feeding cages. Ala. Mar. Res. Bull. 8:25-31.

Table 1. Summary of parasite examinations of Pompano, *Trachinotus carolinus*

	Percentage of fish infested					Modal intensity of infestation*				
	Pre-Culture	Post-culture				Pre-Culture	Post-culture			
		Average	Cage		Pond		Average	Cage		Pond
			Dylox	Formalin				Dylox	Formalin	
Skin Parasites										
Scyphidia	100	87	70	90	100	H	H	H	VL	H
Trichodina	10	60	70	90	20	VL	H	H	VL	L
Gill Parasites										
Scyphidia	100	23	30	20	20	H	H	L	H	VH
Trichodina	10	100	100	100	100	VL	VH	VH	VH	VH
Monogenea	0	43	50	40	20	-	L	L	VL	VL
Alimentary Parasites										
Cestoda	100	0	0	0	0	H	-	-	-	-
Digenea	70	20	20	40	0	VL	L	VL	L	-
Mesentary Parasites										
Nematoda	0	8	10	10	0	-	VL	VL	VL	-
Blood Parasites										
Trypanosoma	0	33	0	0	100	-	H	-	-	VH
	Maximum intensity of infestation*					Percentage with maximum infestation				
Skin Parasites										
Scyphidia	VH	H	H	VH	H	30	43	40	50	40
Trichodina	VL	H	H	H	L	10	30	30	40	20
Gill Parasites										
Scyphidia	H	H	L	H	VH	45	17	20	10	20
Trichodina	L	VH	VH	VH	VH	5	40	40	50	60
Monogenea	-	L	L	L	VL	0	30	40	30	20
Alimentary Parasites										
Cestodes	H	-	-	-	-	70	0	0	0	0
Digenea	L	L	VL	H	-	60	17	20	10	0
Mesentary Parasites										
Nematodes	-	VL	VL	VL	-	0	7	10	10	0
Blood Parasites										
Trypanosoma	-	H	-	-	VH	0	13	0	0	40

* VL - very light infestation (1 to 5 parasites)
L - light infestation (6 to 10 parasites)
M - moderate infestation (11 to 50 parasites)
H - heavy infestation (50 to 100 parasites)
VH - very heavy infestation (100+ parasites)

Table 2. Summary of parasite examinations of striped mullet, Mugil cephalus

	Percentage of fish infested					Modal intensity of infestation[a]				
	Pre-Culture	Post-culture				Pre-Culture	Post-culture			
		Cage			Wild Check		Cage			Wild Check
		Average	Dylox	Formalin			Average	Dylox	Formalin	
Skin Parasites										
Scyphidia	0	10	20	0	0	-	VL	VL	-	-
Trichodina	60	100	100	100	60	VL	VL	L	VL	VL
Copepoda	5	0	0	0	10	VL	-	-	-	VL
Gill Parasites										
Scyphidia	0	10	20	0	0	-	VL	VL	-	-
Trichodina	95	68	95	40	80	M	M	M	VL	L
Monogenea	100	5	0	10	40	M	M	-	M	VL
Copepoda	100	15	30	20	60	VL	VL	VL	VL	M
Alimentary Parasites										
Digenea	60	75	100	50	40	M	VL	VL	VL	M
Kidney Parasites										
Nematoda	5	0	0	0	0	VL	-	-	-	-
Blood Parasites										
Trypanosoma	100	5	10	0	10	M	VL	VL	-	L
	Maximum intensity of infestation[a]					Percentage with maximum infestation				
Skin Parasites										
Scyphidia	-	VL	VL	-	-	0	10	20	0	0
Trichodina	L	M	L	M	VL	10	40	70	10	60
Copepoda	VL	VL	-	-	VL	5	0	0	0	10
Gill Parasites										
Scyphidia	M	M	M	VL	VH	20	45	50	40	10
Trichodina	-	VL	VL	-	-	0	10	20	0	0
Monogenea	M	M	-	M	VL	25	5	0	10	40
Copepoda	M	VL	VL	VL	M	25	10	10	20	10
Alimentary Parasites										
Digenea	M	M	M	M	M	5	35	20	50	20
Kidney Parasites										
Nematoda	VL	-	-	-	-	5	0	0	0	0
Blood Parasites										
Trypanosoma	M	VL	VL	-	L	30	5	10	0	10

[a] VL - very light infestation (1 to 5 parasites)
L - light infestation (6 to 10 parasites)
M - moderate infestation (11 to 50 parasites)
H - heavy infestation (50 to 100 parasites)
VH - very heavy infestation (100+ parasites)

Table 3. Changes in number of parasite types per host*

Species	Sample	% fish with parasite types numbering:		
		0	1 to 3	4 to 8
Pompano	Pre-culture	0	25	75
	Post-culture (av.)	0	40	60
	Cage (av.)	0	40	60
	Dylox-treated	0	50	50
	Formalin-treated	0	30	70
	Pond	0	40	60
Striped mullet	Pre-culture	0	0	100
	Post-culture			
	Cage (av.)	0	60	40
	Dylox-treated	0	50	50
	Formalin-treated	0	70	30
	Wild check	0	50	50
Total	Pre-culture	0	12.5	87.5
	Post-culture	0	50	50

* When the same genera of Protozoa occurred on the gill filaments and on the skin it counted as different types

CASE REPORT OF MULTIPLE PARASITIC INFESTATION AND BACTERIAL INFECTION OF THE SOUTHERN HAKE, Urophycis floridanus[1]

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A southern hake, Urophycis floridanus, was taken on March 22, 1973, from the north jetty sand flats that are associated with the Galveston Bay system, Texas. The hake measured 165 mm total length and was an immature male. The live animal appeared to be healthy and was placed in a 37 liter aquarium for display purposes. Abnormalities noted in the hake on April 4, 1973, included tremors, disorientation, cloudiness of the corneas, and exopthalmia. Although disoriented, the hake was quite lively and exhibited strenuous escape behavior during handling. The animal's gross appearance was normal, except for an excessive amount of mucous, particularly on the gills.

A portion of the gills was removed and a wet mount was prepared. From microscopic examination it was determined that the gills were heavily infested with the parasitic marine protozoan, Cryptocaryon irritans. As many as 92 organisms per field (X100) were counted on the gill tissue.

The abdominal cavity was opened using aseptic procedures. No hemorrhaging was observed; however, the kidney appeared discolored and swollen. Other internal organs appeared normal. A portion of the kidney was removed, and smear slides that were heat fixed and stained with Gram's stain showed an abundance of gram negative rods. Tissue impressions from the kidney were made on tryptic soy agar (Difco Laboratories) with two percent salt added. These soy agar plates were incubated at 28 C. Within 24 hours, colonies of a brownish to yellowish bacterium had swarmed over the surface of the plates. One organism was isolated in pure culture from the initial

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[1] Contribution No. 390, National Marine Fisheries Service Gulf Coastal Fisheries Center, Galveston Laboratory, Galveston, Texas 77550

growth and was identified as Vibrio alginolyticus by Dr. D.H. Lewis of the School of Veterinary Medicine at Texas A&M University.

Tissue specimens for histological examination were taken from the gill, liver, pancreas, cecum, and stomach. The fixative used was 10 percent phosphate-buffered formalin. The tissue specimens were then routinely prepared for paraffin embedding and were sectioned at six to eight microns. All sections were stained with hematoxylin and eosin.

Microscopic examination of the liver revealed an advanced hydrophic or fatty degeneration (Fig. 1a). Few normal hepatocytes were present with most having large cytoplasmic vacuoles. Histologically, the pancreas, cecum, and stomach appeared normal; however, the ceca were virtually filled with a cestode, Tetraphyllidea: Phyllobothriidea. (Fig. 1b). These cestodes possess a terminal myzorhynchus (Fig. 1d) and are probably adults of Echeneibothrium variabile van Beneden, 1850 (personal communication, W. J. Wardle, Texas A&M University, Department of Marine Sciences, Galveston, Texas). As many as 25 sections of the cestode were counted in the lumen of the cecum shown in Fig. 1d. Although many sections examined showed portions of the digestive epithelium engulfed into the bothridia of the cestode, there was no evidence of inflammatory or cellular host response. In addition, there were several larval nematodes, probably Constracaecum sp., present in the mesentery associated with the stomach and cecum (Fig. 1e). In contrast to the cestodes, several of the larval nematodes had evoked an inflammatory response and were being encapsulated.

Fairly advanced stages in the development of Cryptocaryon irritans were observed in association with the gill tissue. Several of the ciliates were located within the gill tissue but most were situated externally between gill lamellae (Fig. 1c). There was little evidence of gill tissue damage attributable to the specimens of Cryptocaryon irritans located externally; however, those within the tissue caused histological damage as evidenced by inflammatory response observed in prepared sections.

According to Sindermann (1970), the ciliate parasite, Cryptocaryon irritans, is the marine counterpart of the ubiquitous freshwater protozoan Ichthyophthirius multifilis, and heavy infections have rarely been seen in natural populations. In aquaria, however, few marine fish species have been found to be resistant to infection. Nigrelli and Ruggieri (1966) reported the pathology of Cryptocaryon to include excessive production of mucous by the host, petechial lesions on the body and gills, erosion of gill tissue, and blindness. Heavy infections were fatal to all hosts.

This southern hake, Urophycis floridanus, apparently was heavily infested with an adult cestode, Echeneibothrium variabile, and a larval nematode, Contraceacum sp., when collected but showed no out-

ward signs of infection. After 13 days in a marine aquarium environment, however, the animal exhibited definite signs of disease. Had it not been for the infection with the ciliated protozoan normally associated with aquaria, the internal infestation of cestodes and larval nematodes would probably have been overlooked.

Vibrio alginolyticus is a common gram negative rod-shaped bacterium that is potentially pathogenic to marine organisms (Lightner and Lewis, in press). The mode of infection of the hake by the bacteria is unknown, but the erosion of the gill tissue by Cryptocaryon may have provided the portal of entry.

LITERATURE CITED

Lightner, D. V., and D. H. Lewis. (In press). Preliminary notes on a septicemic bacterial disease syndrome of penaeid shrimp. Fishery Bulletin.

Nigrelli, R. F., and G. D. Ruggieri. 1966. Enzootics in the New York Aquarium caused by Cryptocaryon irritans Brown, 1951 (Ichthyophthirius marinus Sikama, 1961), a histophagous ciliate in the skin, eyes, and gills of marine fishes. Zoologica 52(3):97-102.

Sindermann, C. J. 1970. Principal diseases of marine fish and shellfish. Academic Press. New York. 369 p.

Figure 1. (opposite page)

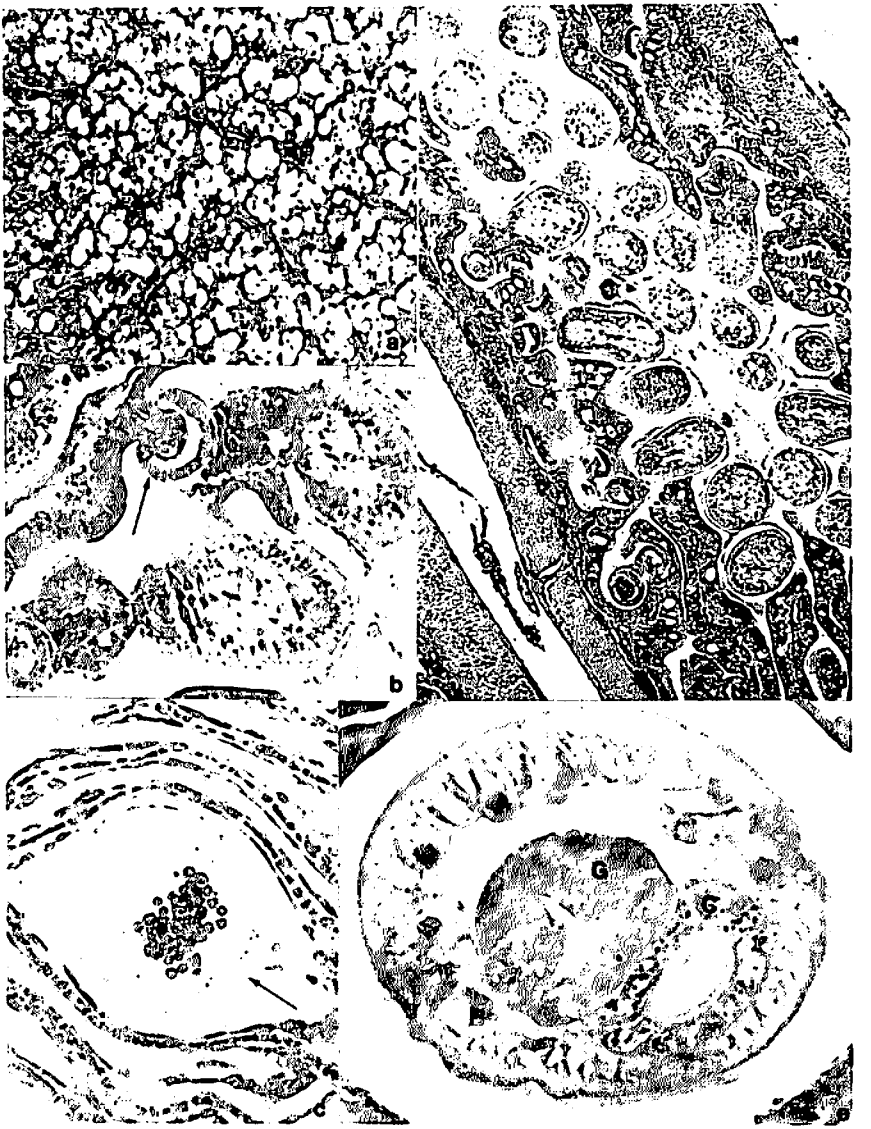
a. Micrograph of the liver showing an advanced hydrophic or fatty degeneration. (X410) (H & E)

b. An adult cestode, Echeneibothrium variabile, with portions of the digestive epithelium enclosed in a bothridia. (X410) (H & E)

c. Histological section of gills showing the parasitic ciliated protozoan, Cryptocaryon irritans. (X410) (H & E)

d. A cross section of a cecum with 25 or more sections of Echeneibothrium variabile visible in the lumen. (X165) (H & E)

e. A cross section of a larval nematode in a mesentary. The parasite is probably a Contracaecum sp. evidenced by (G) a gut with (C) a diverticulum or caecum. (X820) (H & E)


a
b
G
G

IN VITRO CULTIVATION OF CELLS FROM THE SILVER PERCH, Bairdiella chrysura: A SUBSTATE FOR LYMPHOCYSTIS REPLICATION

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INTRODUCTION

Lymphocystis is a disease of viral etiology affecting the higher orders of teleosts. The virus produces tumor-like lesions in the skin and fin areas of both marine and freshwater species and infected cells enlarge up to 10 to 100 times normal size. Normal cell diameters increase from 10-15μ to 200-3000μ in infected cells.

The virus has an icosahedral symmetry, contains DNA as its nucleic acid complement, and is filterable, glycerol sensitive, and tentatively classified as a iridovirus. Electron micrographs show that, intracellularly, the virus replicates in the cytoplasm having a mean diameter of approximately 200mμ. Lymphocystis can be preserved by lyophilization, dessication, or freezing.

This study concerned the development of a system for the in vitro study of a new strain of lymphocystis virus which appeared along the Mississippi Gulf Coast in 1972, as reported by Lawler et al. (1974) of the Gulf Coast Research Laboratory, Ocean Springs, Mississippi.

The study of lymphocystis disease in silver perch comprised the central theme of this invesitgation and included three basic objectives:

1. Establishment of a substrate for in vitro replication of the virus. For this purpose, a cell line was initiated from swim

bladder cells of the silver perch and subsequently labeled SPSB (Silver Perch Swim Bladder).

2. Demonstration of an interaction between the lymphocystis virus and SPSB cells.

3. Study of the in vitro pathogenicity and biochemistry of lymphocystis virus and reinfection of fish with material passaged in cell culture.

RESULTS AND DISCUSSION

At present, there is no suitable established cell line which is available for lymphocystis propagation. Wolf and Quimby (1973) have reported that the BF-1 (Bluegill fry) line and a line from the largemouth bass (LBF-1) will support lymphocystis replication; however, low passage material is required. BF-1 and LBF-1 are not available from ATCC at the required low subculture level.

Thus, our laboratory began and is continuing to maintain SPSB. The cell line is now in its 18th passage, is generally fibroblastic in nature, and is cultured in a medium containing L-15, 20 percent serum, 0.140M NaCl, $CaCl_2$, non-essential amino acids, and antibiotics (Figure 1). Cells exhibit a low affinity for glass, grow best at 26 C, and are subcultured weekly using 0.25 percent trypsin at a split ratio of 1:2. SPSB is unusual in that it will proliferate in either an open or a closed system; however, mortality of planted cells is higher using the open petri dish method.

Karyoligical studies were performed on 9th pass cells using the colcemid metaphase arrest technique. Of 53 cells counted, the highest frequency of occurrence was 19 cells having 48 chromosomes each. Distribution of chromosome numbers ranged from 29 to 50. The karyological pattern consisted of arocentric chromosomes with the exception of one possible metacentric pair, as indicated in Figure 2.

Initial attempts at challenging SPSB with cell free tumor suspensions of silver perch lymphocystis began in the latter part of 1973 using 7th pass cells. Enlarged cells were present in low numbers after 14 days incubation and were attached to the cell monolayer, whereas control flasks did not contain enlarged cells.

Lymphocystis cells increased in size and number as the incubation time was continued. On the 90th day post-inoculation the infected flasks were terminated and stained using the May-Greenwald Giemsa (MGG) method or were photographed in the living state (Figures 4 and 5).

Subsequent investigations demonstrated that infected flasks could be frozen and thawed for the release of lymphocystis virus and that cell free filtrate of thawed material could initiate an

identical response in uninoculated SPSB cells. Thus far, the virus has been passaged three times in SPSB cells.

As the cell line has progressed upward in passage number, the time interval between inoculation and visual observation of enlarged cells has decreased. At passage 12, the virus produced enlarged cells in only eight days. The visual progression, of the development of a virus infected cell begins with the loss of sharp detail of the fibroblastic cell membrane as noted in Figure 3. The cell enlarges, and the increase in size continues as the interval of incubation continues. Usually, the enlarged cell detaches from the monolayer and floats free in the suspending medium as seen in Figure 6.

Eventually, the process of infection produces such a multitude of enlarged cells that the bottle appears to contain "tumor" masses. Gross visual observation of infected versus non-infected cells demonstrates the increased opacity of the infected bottle, and many such foci (Figure 7) of infection are evident.

The nuclei of infected cells which have detached from the monolayer have diameters of approximately 26μ. This contrasts with normal nuclear diameters of approximately 9μ, representing a 2.8X increase in size.

Cells infected in vitro have been consistently smaller than naturally infected cells. In fact, in vitro lymphocystis infection of SPSB does not simulate an overwhelming infection wherein the entire monolayer is destroyed but it does establish an equilibrium whereby an infected bottle can be monitored for at least four months. Replacement of the growth media on a weekly basis removes the floating cells, and because the process of enlargement is extremely slow, uninfected cells replicate freely, filling in spaces vacated by detached cells. This creates a continually renewable substrate for viral replication.

Since it had previously been reported (House and Christmas, 1971) in vivo lymphocystis tumor cells contain discrete virus particles, it was pertinent to this investigation to demonstrate that the infected SPSB cell did in fact contain the same type of particle. Thus, floating cells were recovered by careful centrifugation and then fixed and stained for electron miscroscopy.

Virus particles were found in the cytoplasm of infected cells as shown in the next two figures. Figure 8 represents an interface of two infected cells containing numerous lymphocystis particles at a magnification of 6,525. At magnification of 38,280 (Figure 9), the same tissue culture preparation shows enlarged particles with an approximate diameter of 250-300nm.

To date, two of the four postulates used to characterize an

infectious agent have been completed. Upcoming studies include inoculation of silver perch with material passaged *in vitro* and reisolation of the virus from experimental infections.

SUMMARY

In summary, SPSB appears to be an excellent substrate for lymphocystis replication. At this time, cells respond to virus infection, and microscopic differentiation of infected versus non-infected cells is a relatively simple matter. As with BT-1, SPSB may become resistant to infection once it reaches higher subculture levels, and low pass material may be required to continue such studies as viral synthesis, tumor immunology, or tumor biochemistry on this particular virus.

ACKNOWLEDGEMENTS

This investigation was funded by University Research Grant #2609 and in part by funds from the National Oceanic and Atmospheric Administration, Sea Grant Program. The authors wish to extend their appreciation to Dr. Harold Howse and his staff of the Gulf Coast Research Laboratory for their preparation of the excellent electron micrographs employed in this study.

LITERATURE CITED

Howse, H. D., and J. Y. Christmas. 1971. Observations on the ultrastructure of lymphocystis virus in the Atlantic croaker, *Micropogon undulatus* (Linneaus). Virology 44:211-4.

Lawler, A. R., H. D. House, and D. W. Cook. 1974. Silver perch, *Bairdiella chrysura*: new host for lymphocystis copeia. (In press).

Wolf, K., and M. C. Quimby. 1973. Fish viruses: Buffers and methods for plaquing eight agents under normal atmosphere. Appl. Microbiol. 25:659-64.

Figure 1. Normal morphology of SPSB cells; MGG stain. (600X)

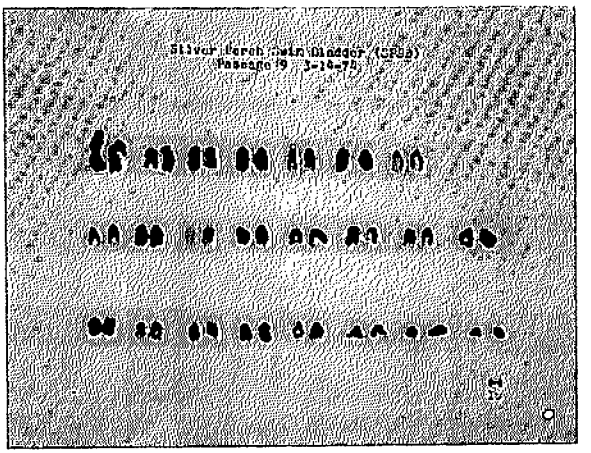

Figure 2. Karyological pattern of SPSB cells - Passage 9.

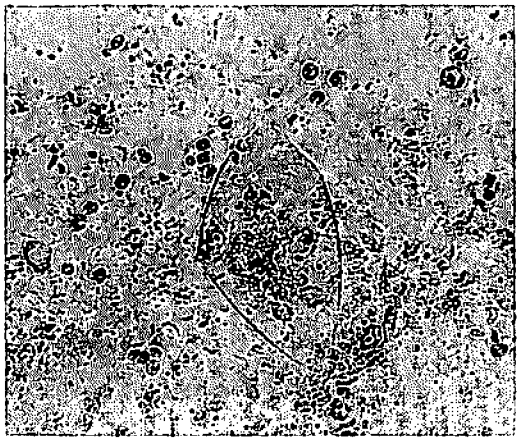

Figure 3. Enlarged lymphocystis cell attached to monolayer; unstained preparation. (383X)

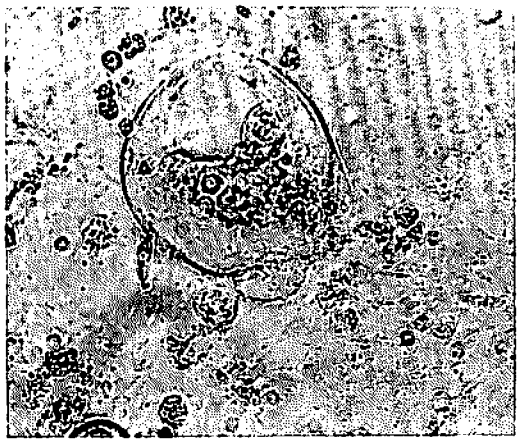

Figure 4. Unstained enlarged cell detached from the monolayer; 90 days post-infection. (720X)

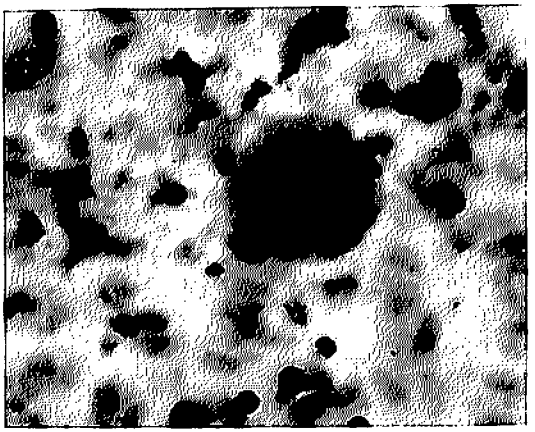

Figure 5. MGG stained enlarged cell detached from the monolayer; 90 days post-infection. (1100X)

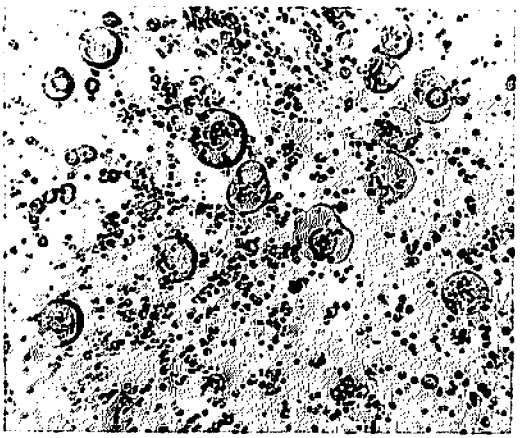

Figure 6. Lymphocystis cells floating free in the suspending medium; living material. (180X)

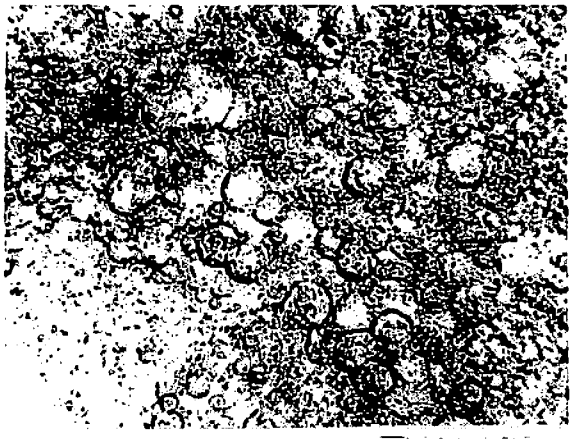

Figure 7. Light microscopic examination of typical aggregate composed of numerous lymphocystis cells; living materials. (140X)

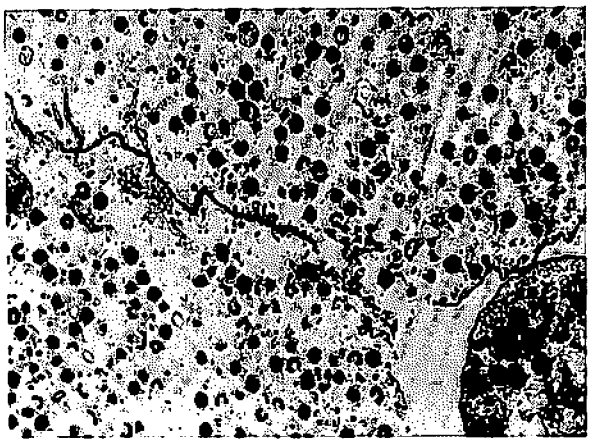

Figure 8. Interface of two lymphocystis infected cells showing discrete virus particles. (6,525X)

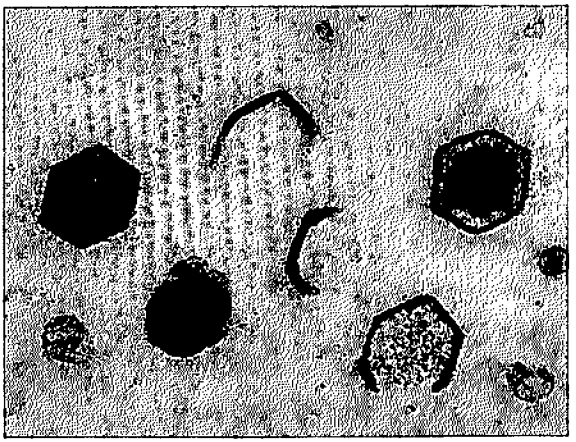

Figure 9. Electron micrograph of individual lymphocystis particles in SPSB infected cells. (38,280X)

BACTERIAL INFECTIONS OF CAGE-CULTURED CHANNEL CATFISH IN BRACKISH WATER[1]

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INTRODUCTION

Many bacteria are indigenous to the aquatic environment, but most are considered to be harmless to fish under normal conditions. Healthy fish are resistant to bacterial infections due to the presence of large amounts of bactericidal substances in the blood which help them to overcome an infection (Van Duijn, 1973). Bacterial epizootics can occur when fish are cultured under intensive conditions, especially when fish are crowded, when dissolved oxygen concentration is low, or when high numbers of bacteria are present in the water (Bullock and Snieszko, 1969). Several aquatic bacteria have been shown to be pathogenic to fresh-water fishes (Meyer, 1964; Rogers, 1970; Van Duijn, 1973), and to marine fishes (Sindermann, 1970; Van Duijn, 1973). Bullock and McLaughlin (1970) give an extensive review of this subject.

The present report is based on observations made during the course of an experiment on high-density cage culture of channel catfish in brackish water (Kilgen, Harris, and Vidrine, 1974) Several

[1] This study was partially funded by Louisiana Land and Exploration Company, and Office of Sea Grant of the National Oceanic and Atmospheric Administration, Department of Commerce.

fish were checked for the presence of bacteria when they exhibited characteristic symptoms of bacterial infection, such as necrotic lesions on the body surface and eroded fins.

MATERIALS AND METHODS

Two brackish-water, oil-field pipeline canals were used in this study. The canals, located in a salt-marsh, estuarine area of Lafourche Parish, Louisiana, are about 3 m deep and 20-25 m wide and are damned at both ends. Each canal has a 76 cm culvert with a gate-valve which allows water control, using water from the surrounding estuarine area.

Canal R-1 (2.55-ha) was rotenoned in December, 1972, in an effort to remove all fishes used in a previous experiment. Canal R-2 (4.45-ha) had been rotenoned in May, 1972, and was subsequently stocked with 618 channel catfish fingerlings per hectare. These were allowed to remain in the canal during the present experiment.

Each canal was stocked with 10 cages (one m^3) of channel catfish, each containing 500 fish (61 g average), between 1-17 March, 1973. The overall stocking rates were 392 per ha for R-1, and 225 per ha for R-2. The cages were constructed of vinyl-coated, 13 x 38 mm mesh size, 16-gauge galvanized steel wire, reinforced by a steel strap, top and bottom. They were cylindrical, had a plywood top with a feeding door, and were evenly spaced along the length of each canal, each cage being secured to a wooden stake by a rope three m long. Styrofoam blocks around the inside provided flotation and also served as a feeding ring to prevent feed from floating out of the cage. The fish were given a daily ration of Purina Floating Cage Catfish Chow ranging from five to two percent of their body weight. The experiment was terminated prematurely, mainly because of poor feed conversion, disease problems, and poor survival.

In an attempt to identify and control the factors causing the mortalities, we harvested the surviving catfish on 13-14 August, 1973 and restocked them into 18 cages ($400/m^3$) in canal R-2. After observing several of the larger fish with lesions during the first two weeks of restocking, we checked several fish for possible bacterial infections. Using the determinative schemes outlined by Lewis (1973) on samples taken from lesions and skin of infected fish, the bacterial isolates were identified.

RESULTS AND DISCUSSION

In the original phase of the experiment, three peak periods of high mortalities occurred in R-1 during the 19 week experiment: mid-May, late June-early July, and late July-early August (Fig. 1). Peak mortalities in R-2 occurred during mid-April, mid-June, and early August (Fig. 1). The only corresponding periods of peak mortality occurred in both canals in early August, although Fig. 1 does reveal that mortalities generally occurred at about the same time in both canals.

During the first and second weeks of the second phase of the experiment, 10 percent of the fish were lost apparently due to bacterial infections (Fig. 2). Examination of several fish showing disease symptoms such as lesions and eroded fins revealed the presence of four different bacterial populations. According to the determinative outline of Lewis (1973), the most numerous organism was a Flavobacterium, the second most numerous was Aeromonas liquefaciens (A. punctata). A third bacterium could not be identified using the outline. It was a gram negative rod, oxidase-positive, non-fermentative, and non-pigmented. Micrococcus halodurans was present in small numbers.

Some flavobacteria are commonly found in fish slime. No references could be found indicating that Micrococcus had been identified as a fish pathogen. It was probably also part of the indigenous flora. Aeromonas liquefaciens has been identified as the causal agent of infectious dropsy of carp (Schaperclaus, 1969) and mortalities of shad (Haley et al., 1967) and golden shiners (Meyer, 1964). Although Ghittino (1972) describes it as "an ubiquitous water bacterium which become virulent to aquatic organisms under various conditions of stress", Caselitz (1966) and also von Graevenitz and Mensch (1968) have shown that it can be also pathogenic to humans. After finding that Aeromonas was present, we prepared a dip treatment of 150 ppm formalin for each cage and then we offered the fish feed treated with 18 g oxytetracycline activity per kg of feed. We did this by dissolving the oxytetracycline in cooking oil, and mixing it with the commercial pelleted feed. We fed them this mixture for 10 days, and mortalities ceased within one week after beginning the treatment. The fish were subsequently harvested on 15 October, 1973. Following the treatments (Fig. 2) we lost only seven fish over the last six weeks of the experiment.

We attempted to pinpoint the reasons for the outbreaks of bacterial infection and subsequent mortalities. According to Van Duijn (1973), "fishes have a very great resistance to disease so long as they are not weakened by bad treatment, unsuitable food, lack of oxygen, too high or too low temperature, or other adverse influences." We considered each of these factors individually, and

also in combination.

Treatment

The cages were removed from the water bi-weekly, during which time the fishes were in a mass on the bottom of the cage and were at times highly agitated. They may have become injured during the process of lifting the cages and also might have scraped themselves on barnacles which grew on the styrofoam floats. In addition, they were also periodically dipped out of the cages using a small net. Any of these activities could have broken the skin on a few individuals, making it possible for indigenous bacteria to invade and multiply within the tissue of the fish, leading to pathogenic, conditions especially since the fish were densely crowded.

Food

The quality of the feed during the course of the experiment was unstable. Some feed failed to float, and some was infested with weevils. The fish sometimes did not get their feed due to nonfloatation; and sometimes got feed of inferior quality. At times, even if the feed did float, the fish would not eat it. The National Research Council Subcommittee on Fish Nutrition (1973) listed 11 vitamins, of which a deficiency could cause poor appetite and poor growth of catfish.

Oxygen

The lowest dissolved oxygen concentration observed at the surface was 3.0 ppm, but it generally ranged from 5.0 to 7.6 ppm. It was noted however, that a complete absence of oxygen on the bottom of the canals occurred on several occasions. Oxygen determinations were usually made at daybreak on a daily basis, and thus probably represent the lowest values. Relatively low oxygen concentration may have caused additional stress.

Temperature

During periods of peak mortality, surface temperatures were not observed over 33 C or lower than 24 C, and never was there an extreme rise or drop in temperature.

Other Factors

Salinity ranged from 4.0 to 6.4 ppt during the first phase but rose to 9.8 ppt during the fourth week of the second phase, during the therapeutic treatment period. This was due to flooding over the levees from the surrounding marsh area. The pH of the canal waters generally ranged from 6.9 to 8.3, with lower values being found on the bottom of the canal.

CONCLUSIONS

Outbreaks of infection and associated mortalities were probably the result of a combination of several of the aforementioned factors. Rough treatment of fish in a crowded condition during warm weather, and in brackish water may have in combination contributed to the infections. Salinity may decrease the natural physiological resistance of the fish to bacterial infections but may have contributed to the reduced incidence of mortalities in September. Unfortunately, the rise in salinity to 9.8 ppt occurred concommittant with treatment for Aeromonas, and thus it could not be concluded that the treatment in itself was the sole cause of the disappearance of bacterial infection. On the other hand, we could not really determine that a rise in salinity of 4.0 to 6.4 ppt contributed to an increase in bacterial infection.

LITERATURE CITED

Bullock, G. L., and J. J. A. McLaughlin. 1970. Advances in knowledge concerning bacteria pathogens to fishes (1954-1968). In A symposium on diseases of fishes and shellfishes (S. F. Snieszko, ed.) Amer. Fish. Soc. Spec. Publ. No. 5. p. 231-42.

Bullock, G. L., and S. F. Snieszko. 1969. Bacteria in blood and kidney of apparently healthy hatchery trout. Trans. Amer. Fish. Soc. 98(2):268-71.

Caselitz, F. H. 1966. Pseudomonas-Aeromonas undihre human-medizinische Bedeutung. Jena: Gustan Fischer Verlag.

Ghittino, P. 1972. The principal aspects of bacterial fish diseases in Italy. In Symp. Zool. Soc. Lond. 30:25-38.

Haley, R., S. P. Davis, and J. M. Hyde. 1967. Environmental stress and Aeromonas liquefaciens in American and threadfin shad mortalities. Prog. Fish-Cult. 29(4):193.

Kilgen, R. H., A. H. Harris, and C. Vidrine. 1974. Problems of fish culture in cages in brackish-water pipeline canals. Proc. World Maricult. Soc. (In Press).

Lewis, D. H. 1973. Predominant aerobic bacteria of fish and shellfish. Texas A & M Univ. TAMU-SG-73-401. 10 p.

Meyer, F. P. 1964. Field treatments of Aeromonas liquefaciens infections in golden shiners (Notemigonus crysoleucas). Prog. Fish-Cult. 26(1):33-5.

National Research Council, Subcommittee on Fish Nutrition. 1973. Nutrient requirements of trout, salmon, and catfish. National Academy of Sciences. Nutrient Requirements of Domestic Animals. No. 11. 57 p.

Rogers, W. A. 1970. A summary of fish disease cases received over a five-year period at the Southeastern Cooperative Fish Disease Laboratory. In Proc. 23rd Ann. Conf. Southeast. Assoc. Game Fish Comm. 1969: 353-8.

Schaperclaus, W. 1969. Virusinfektionen bei Fischen. In Handbuch ber Virusinfektionen bei Tieren (H. Rohrer, ed.). Jena: VEB Gastav Fischer. 52:1067-141.

Sindermann, C. J. 1970. Principal diseases of marine fish and shellfish. Academic Press, New York. 369 p.

Van Duijn, C. 1973. Diseases of fishes. Charles C. Thomas. Springfield, Illinois. 372 p.

von Graevenitz, A., and A. H. Mensch. 1968. The genus Aeromonas in human bacteriology. New Engl. J. Med. 278:245-9.

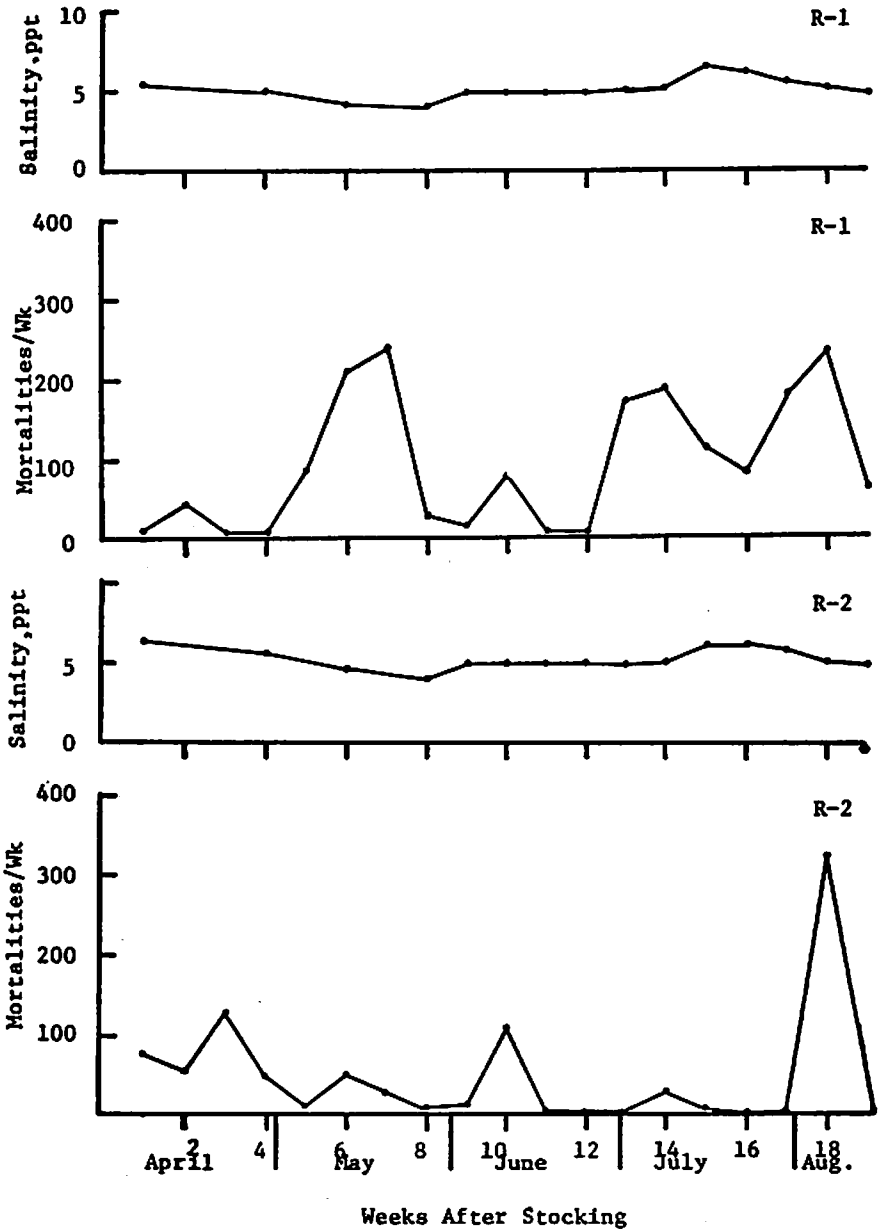

Figure 1. Observations on mortalities and salinities in canals R-1 and R-2, April 1-August 13, 1973. Each canal was stocked with 10 cages, each containing 500 channel catfish.

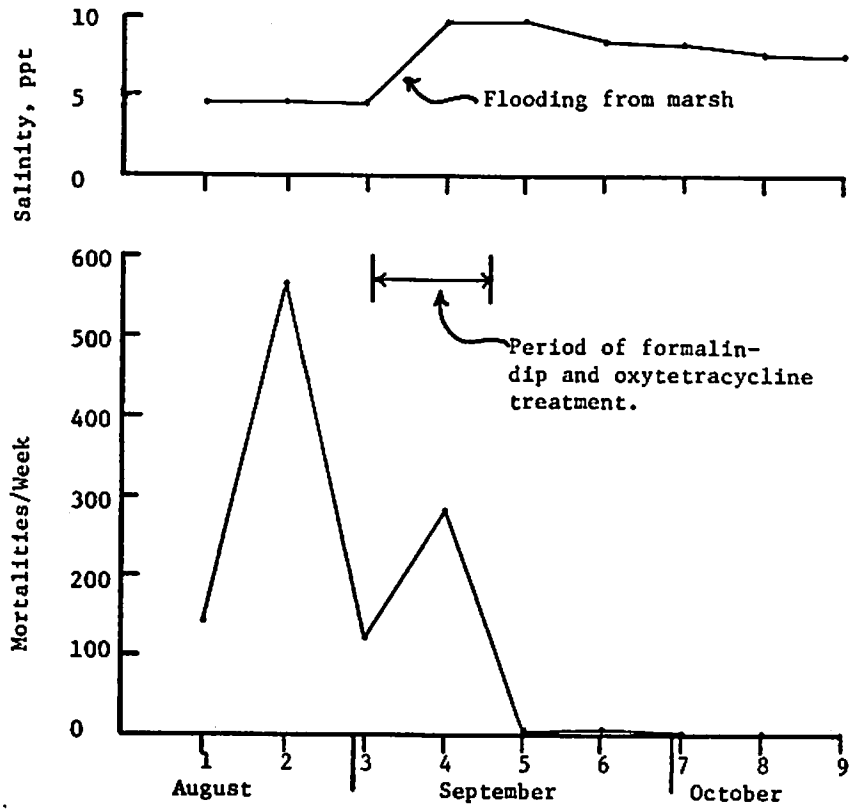

Figure 2. Observations on mortalities and salinities in canal R-2, August 14-October 15, 1973. Channel catfish were stocked in 18 cages, each containing 400 catfish.

OBSERVATIONS ON THE INCIDENCE OF SHELL DISEASE IN SOUTH CAROLINA BLUE CRABS, *Callinectes sapidus* (RATHBUN)[1]

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INTRODUCTION

Shell diseases have been reported in several commercially important marine crustaceans, including the American lobster (*Homarus americanus*), penaeid shrimp (*Penaeus aztecus*, *P. duorarum* and *P. setiferus*), king crabs (*Paralithodes camtschatica* and *P. platypus*), and blue crabs (*Callinectes sapidus*) (Hess, 1937; Sawyer and Taylor, 1949; Rosen, 1967, 1970; Sindermann and Rosenfield, 1967; Mahood et al., 1970; McKenzie, 1970; Cook and Lofton, 1973). Necrotic exoskeletal lesions which characterize the disease often superficially resemble cigarette burns, hence the common name "burned spot" disease.

Although the causative agents of shell disease have not been positively identified, strong evidence suggests that the culprits are chitinoclastic bacteria (and perhaps fungi) which invade abraded and damaged areas of the exoskeleton (Rosen, 1967, 1970; Cook and Lofton, 1973). The first step in the infection probably is mechanical abrasion or injury of a crustacean's exoskeleton by sand, sharp objects or other animals. Then the inner

[1]Contribution No. 16 from the South Carolina Marine Resources Center. This study was supported by Contract No. 10340034 from the Coastal Plains Regional Commission and by the State of South Carolina.

layers of the shell are opened to attack by chitinoclastic bacteria which are ubiquitous in marine and estuarine waters and which form part of the normal external flora of marine crustaceans. The bacteria multiply in the abrasions and wounds where they digest the chitinous portion of the shell and in the process produce acids which dissolve the calcified portions. Dissolution of the shell may continue until all layers are breached. However, the disease apparently does not penetrate into the internal organs. Only by ecdysis can an animal rid itself of the disease. Thus, it is not surprising that older crustaceans which molt infrequently, if at all, are most affected.

In its early stages the disease may appear only as a few small brown or brownish-red spots which resemble puncture wounds. In later stages, groups of these spots may coalesce to form large irregular lesions which are usually deepest at their centers. The mid-dorsal surface of the carapace and the lateral spines of blue crabs are frequently the locations of large lesions. Some heavily infected crabs may have one or more large "burns" but exhibit few if if any of the early stage "pockmarks". In such cases the lesions probably resulted from secondary infection of substantial wounds.

Since the disease infects only the shell of the animals, it has no effect on their edibility. However, animals with advanced infections are certainly more susceptible to death during capture, handling, and molting than are uninfected crabs, and thus they are less valuable to crab fishermen. Also, the disease is contagious, and it may pose a threat for blue crab shedding operations (i.e. commercial soft crab production) where large numbers of crabs are crowded together in floats or tanks (Rosen, 1967, 1970: Cook and Lofton, 1973). An increased incidence of the disease under these conditions would not be surprising in view of the numerous instances of mechanical damage which occur in shedding operations. Therefore, a survey of the incidence of the disease in various areas of the state was undertaken as part of the first phase of an investigation concerning the feasibility of establishing a soft blue crab industry in South Carolina. A study of the occurrence of the infection in the commercial crab catch was conducted concurrently as part of a state-supported crab population monitoring program.

METHODS AND MATERIALS

The data reported in this paper were obtained from two sources: (1) a field survey of blue crabs and (2) a commercial catch sampling program. These two studies were conducted independently, and the methods used are described separately below. In the field survey, attempts were made to note all occurrences of

shell disease. However, since examinations for shell disease were conducted incidental to other observations, it is probable that many light infections went undetected. In samples from the commercial catch, note was take only of those crabs with large, readily apparent shell lesions or extensively pitted exoskeletons.

Statistical analyses of the data were performed using a Hewlett-Packard model 9810A electronic calculator.

Field Survey Methods

Four regular sampling stations were located in blue crab fishing areas along the South Carolina coast as follows (See Fig. 1):

1) in Five Fathom Creek near McClellanville (33°01.8'N Lat., 79°28.5'W Long.),

2) in the Stono River near Charleston Harbor (32°40.5'N Lat., 79°59.6'W Long.),

3) in the Ashepoo River near St. Helena Sound (32°33.0'N Lat., 80°27.4'W Long.),

4) in the Beaufort River near Port Royal Sound (32°20.1'N Lat., 80°40.5W Long.).

Sampling began in April 1973, and each station was visited at approximately two-week intervals through October and then monthly from November through March 1974. Upon arrival on station, ten standard four-funnel crab pots were baited and set. These pots were allowed to fish for six hours. Immediately after the pots were set a hydrographic cast was made. This consisted of measuring temperature and taking water samples for determination of salinity and dissolved oxygen concentration at one meter below the surface and just above the bottom. Also, light penetration was measured with a Secchi disc. Another hydrographic cast was taken immediately before the pots were retrieved, and one or two others were taken during the intervening six hours in conjunction with other sampling at the same station.

The blue crab catch from each pot was placed in a separate plastic bag or covered plastic garbage pail marked with the appropriate collection information. Samples were returned to the laboratory where they were placed in cold storage until they could be processed. In the laboratory, each crab was examined individually and the following data were recorded: sex, state of maturity, evidence of approaching or recent molt, "long" (spine to-spine) width, "short" width (width just anterior to spines),

carapace length, total weight, obvious disease symptoms, absence of one or both chelae, presence of large fouling organisms (such as barnacles) or other external commensals (for example, leeches), and any deformities or major injuries.

Commercial Catch Sampling Methods

In South Carolina the major portion of the crab fishery is located in the southern part of the state in the vicinity of St. Helena, Port Royal, and Calibogue Sounds (Fig. 1). Most of the crabs caught by fishermen in this region are sold to a single processor, Blue Channel Corporation, which is located at Port Royal (Fig. 1). This firm processes some 50 per cent or more of the annual South Carolina blue crab catch.

Periodic sampling of commercial catches sold to Blue Channel Corporation was initiated in August 1972 to obtain data relating to the management of South Carolina's blue crab stocks. Catches generally were sampled once weekly between May and November, and about once a month during the rest of the year. Most of the crabs were captured in pots. However, a small trawl fishery for crabs exists in Port Royal Sound from December to March. A number of crabs from this fishery, which consists predominately of mature females, were sampled. Usually, a sample of 50 crabs from each fisherman who brought his catch to Blue Channel Corporation was examined. However, larger samples were occasionally taken, particularly when a trawler unloaded its catch. The sex, carapace width, date and location of capture, type of gear, presence or absence of shell lesions or extensive pitting of the shell, and name of the fisherman were recorded for each sample. Observations on the incidence of shell disease were initiated in January 1973.

Biases in the Data

The data reported are subject to several biases and limitations:

1) Observations were limited for the most part to crabs of legal size, five inches (127 mm) from tip to tip of the lateral spines. Only approximately 5 per cent of the commercially caught crabs examined were less than 127 mm wide. Similarly, the standard crab pots employed in the field survey caught primarily adults and large juveniles. Thus, small crabs were not sampled.

2) The taking of ovigerous females by fishermen is illegal in South Carolina, so in the commercial catch no ovigerous crabs were available for examination.

3) Many fishermen cull the large male crabs from their catches before bringing the animals to Blue Channel Corporation. These large males are sold separately for shipment to markets in Virginia and Maryland. Thus, the average size of crabs in the commercial sample was somewhat less than that in the exploited population.

4) Fewer samples were taken in winter when the disease appeared more prevalent than in summer when incidence was generally lower.

5) While attempts were made to record all instances of shell disease among crabs captured in the survey, only those with open lesions or extensive pitting of the carapace were noted in the commercial sample. Thus, the latter observations do not indicate the true incidence of the disease in the exploited population.

RESULTS AND CONCLUSIONS

Field Survey

Mean (plus or minus one standard deviation) temperatures and salinities are summarized by months for the survey stations in Table 1. Temperatures were similar at all stations, and salinities at the McClellanville, Stono, and Beaufort stations were high and generally stable. However, the Ashepoo salinity regime varied from oligohaline to polyhaline conditions.

The number of crabs collected at each station and the incidence of shell disease in these samples are summarized by months for juveniles and adults of both sexes in Table 2. The prevalence of shell disease in these collections indicate that it is a common and widespread infection of blue crabs in South Carolina. In fact, only on one occasion (Ashepoo station, December 1973) were no infected crabs captured (Table 2).

Monthly infection levels ranged from 0 to 53.1 per cent, depending on the station, and the disease was generally much more prevalent during late fall and winter than during warmer months. (Table 2). This trend was evident at all locations, though less obvious at the Ashepoo station, and became particularly apparent when data from all stations were combined (Table 3). A Chi-Square test showed that differences in overall incidence levels between months were highly significant (Chi-Square value = 247.84**, df = 11, P less than 0.005). That the periods of high incidence of shell disease and low temperatures coincide (Table 1) is not surprising since such temperatures inhibit molting.

A Chi-Square test also indicated differences in annual infection levels between locations (Chi-Square value = 60.99**, df = 3, P less than 0.005). The highest overall frequency (14.2 per cent) occurred at the Stono River station. This level was over 1.5 times greater than the next highest incidence, which occurred at the Beaufort River station (Table 3, Fig. 2). No clear relationship between these levels of infection and hydrographic conditions at the stations (Table 1) is apparent. However, the lowest annual incidence (6.6 per cent) was recorded at the Ashepoo River station which exhibited by far the least stable salinity regime and the lowest salinities.

Juveniles of both sexes exhibited much lower infection levels than adults. This is not surprising since juveniles molt more frequently than do adults (adult females do not molt at all), and their exoskeletons are thus less likely to become seriously infected by shell-destroying bacteria.

The prevalence of shell disease was significantly greater among ovigerous crabs than among non-ovigerous adult females (Table 4) (Chi-Square value = 53.35**, df = 1, P less than 0.005). In many cases the ovigerous crabs may have been longer in their terminal anecdysis than non-ovigerous ones. The longer the intermolt period, of course, the more likely the occurrence of some shell damage and subsequent invasion of the injured area by chitinoclastic bacteria. Also, the large, cumbersome egg masses certainly interfere with the mobility of the crabs and thus may increase the probability of shell injury. Finally, all ovigerous crabs caught by fishermen by law must be returned to the water. Undoubtedly, many receive injuries in the process of capture and handling, and when they are released these injuries become points of invasion for the omnipresent chitin-destroying bacteria.

When all collection data were lumped by sex (Table 4), it was found that the total incidence of shell disease in males (11.2 per cent) was about twice that in females (5.3 per cent). A Chi-Square test indicated that this difference was highly significant (Chi-Square value = 55.43**, df = 1, P less than 0.005). This result is surprising since there is little a priori reason to expect that males would be more susceptible to an external shell infection than females. In fact, adult females might appear to be the more likely candidates for the disease since they do not molt after attaining maturity while adult males do, albeit infrequently. On the other hand, males are generally more aggressive than females and perhaps may be injured more frequently. Also, the sampling procedure did not result in capture of equal numbers of males and females. At the Beaufort station females out-numbered males about 1.2:1 but at other stations the ratio of males to females ranged from about 2 to 5.1. Thus, although the difference in infection level by sex appears to be significant, it may

only be apparent because of sampling bias or some location effect. This question will be considered again below with regard to the commercial catch data.

Commercial Catch Survey

Commercial catches of blue crabs from the North Edisto River and the St. Helena, Port Royal, and Calibogue Sound areas (Fig. 1) were sampled at Blue Channel Corporation. Data from these areas emphasize the ubiquity of the shell disease among South Carolina blue crabs, since some infected speciments occurred in nearly all the samples (Table 5).

Infection levels varied considerably from sample to sample, location to location, and with sex at different locations (Table 5). Although incidence of the disease was less than five per cent in most samples, it reached 19.6 per cent in Port Royal Sound during one period (22 November to 31 December 1973). This suggests that the disease may be quite prevalent in some areas during late fall and early winter.

Information from the commercial catch also indicates, though less domonstrately than do the field survey data, that the level of infection varies seasonally. The observed incidence was generally greatest during the period from October 1973 to January 1974. This trend was most evident in the St. Helena and Port Royal Sound regions (Table 5). However, rather high infection levels also occurred in some locations in July but never reached the wintertime peaks.

When all observations for each major fishing area were summarized (Table 6), it became evident that incidence of shell disease varied considerably between sexes and among areas. A Chi-Square test showed these differences to be highly significant of each sex (males: Chi-Square value = 46.55**, df = 3, P less than 0.005; females: Chi-Square value = 12.44**, df = 3, P less than 0.01) and for both sexes combined (Chi-Square value = 24.54**, df = 3, P less than 0.005). Thus, it appears that blue crabs in the Port Royal Sound area are more likely to be affected by shell disease than those in other major fishing areas (Table 6). However, this conclusion must be considered provisional since the samples were from commercial fishermen and did not represent the entire population. Also, no explanation for such differences is readily apparent.

In contrast to the field survey findings, the total incidence was observed to be the same for both sexes when all data from all locations were combined (Table 6). This finding is based on examinations of nearly 11,000 crabs from a wide area and range of

habitats, not just from a few fixed station. However, although these data suggest that the total infection rate may not vary between sexes, it is evident that local differences in infection may exist (Tables 2 and 6).

SUMMARY AND GENERAL DISCUSSION

According to Rosen (1967), "Our knowledge of the shell disease . . . is limited mainly to impounded commercial crustaceans." Previous reports of the disease in wild blue crab populations are limited to those of Mahood et al. (1970) and McKenzie (1970) which dealt with the same collections. During a study of the causes of mass blue crab mortalities in the South Atlantic, Mahood et al. (1970) found that 28 (2.33 per cent) of a total of 1200 blue crabs examined were infected with shell disease, some quite heavily. Twelve of these specimen (all males) were taken from high salinity South Carolina waters during winter (McKenzie, 1970).

In the present study, some symptoms of shell disease were observed in 535 (9.2 per cent) of a total of 5,824 crabs examined during the field survey, while 371 (3.4 per cent) of a total of 10,940 crabs sampled from the commercial catch exhibited large shell lesions or extensive pitting of the exoskeleton. The latter figure is probably a good estimate of the level of heavy infections in the exploited population.

Several other generalizations concerning the occurrence of shell disease among South Carolina blue crabs may be made:

1) The disease was more prevalent during late fall and winter than during summer.

2) Incidence of the disease varied significantly between locations. The reason for this variation is unknown, although it may be related in small part to salinity fluctuation.

3) The disease primarily affected adult animals.

4) Ovigerous crabs exhibited a significantly higher infection level than did non-ovigerous adult females.

5) The field survey data suggested that males were more susceptible to shell disease than females. In contrast, information from the commercial catch indicated that there was no difference in total incidence between sexes, though differences did occur in specific areas. The latter observation probably explains why the infection rates of males and females appeared to differ between the two sets of data.

While animals with shell infections may live for several months or may rid themselves of the disease through ecdysis, serious consequences of such infections are possible:

1) The disease itself may be fatal. Sawyer and Taylor (1949) have stated that shell disease may destroy the chitinous layer of gill filaments in lobsters and thus interfere with respiration. McKenzie (1970) reported observing a crab whose carapace was so badly eroded by shell disease that gill filaments were visible. We have observed similar cases. Reports from reliable fishermen and our own observations indicate that such heavily infected crabs often are weak, lethargic, and die rapidly when removed from the water.

2) The disease is contagious (Sindermann and Rosenfield, 1967; Rosen, 1970; Cook and Lofton, 1973), and recently molted crabs appear especially susceptible to infection since their soft exoskeletons may be damaged easily.

3) While the shell disease itself apparently does not penetrate to internal tissue (Rosen, 1967, 1970; Cook and Lofton, 1973), it may provide opportunities for the invasion or multiplication of other more virulent forms. Of special importance in this context is Vibrio parahaemolyticus, a pathogen involved in certain types of serious food poisoning in humans. Krantz et al. (1969) isolated V. parahaemolyticus from moribund blue crabs in Chesapeake Bay shedding tanks, and Colwell (1970) reported the pathogen to be endemic in water, sediments, and animals in estuarine environments. She stated, "Healthy crabs and oysters harbor the organism in very low but detectable numbers. When the animal is weakened, either by infection, predation, or through environmental changes, this organism can invade swiftly and fatally." She then concluded that, ". . . being a salt requiring marine bacterium [V. parahaemolyticus] is primarily a potential pathogen of marine and estuarine animals and only secondarily a human pathogen." The possibility that shell disease may provide an opportunity for the invasion of V. parahaemolyticus and that this pathogen may then result in widespread blue crab mortalities or be transmitted to human populations should be carefully examined in future studies.

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LITERATURE CITED

Cook, D. W., and S. R. Lofton. 1973. Chitinoclastic bacteria associated with shell disease in Penaeus shrimp and the blue crab (Callinectes sapidus). J. Wildl. Dis. 9:154-9.

Colwell, R. R. 1970. Computer studies of microorganisms associated with invertebrate animals in the Chesapeake Bay and isolation of Vibrio parahaemolyticus from the blue crab (Callinectes sapidus). p. 175-92, In Food-Drugs from the Sea, Proceedings 1969 (H. W. Youngken, Jr., ed.). Marine Technology Society. Washington, D. C.

Hess, E. 1937. A shell disease in lobsters (Homarus americanus) caused by chitinovorous bacteria. J. Biol. Bd. Canada, 3(4): 358-62.

Krantz, G. E., R. R. Colwell, and E. Lovelace. 1969. Vibrio parahaemolyticus from the blue crab Callinectes sapidus in Chesapeake Bay. Science, 164 (3885): 1286-7.

Mahood, R. K., M. D. McKenzie, D. P. Middaugh, S. J. Bollar, J. R. Davis, and D. Spitzbergen. 1970. A report on the cooperative blue crab study--South Atlantic states. Georgia Game and Fish Commission. Coastal Fish. Div. Contribution 19. 32 p.

McKenzie, M. D. 1970. Fluctuations in abundance of the blue crab and factors affecting mortalities. South Carolina Wildl. Resour. Dept. Mar. Resour. Div. Technical Report 1. 45 p.

Rosen, B. 1967. Shell disease of the blue crab, Callinectes sapidus. J. Invert. Pathol. 9(3):348-53.

Rosen, B. 1970. Shell disease of aquatic crastaceans. In A Symposium on Diseases of Fishes and Shellfishes (S. F. Sniezko, ed.). Amer. Fish. Soc. Spec. Publ. No. 5. Washington, D. C. p. 409-15.

Sawyer, W. H., Jr., and C. C. Taylor. 1949. The effect of shell disease on the gills and chitin of the lobster (Homarus americanus). Maine Dept. Sea and Shore Fish. Res. Bull. No. 1. 10 p.

Sindermann, C. J., and A. Rosenfield. 1967. Principal diseases of commercially important marine bivalve Mollusca and Crustacea. Fish. Bull. 66:335-85.

Table 1. Monthly temperatures and salinities ($\bar{x} \pm s_d$) at survey stations (surface and bottom determinations combined)

Month	McClellanville		Stono		Anhepoo		Beaufort	
	Temperature (°C)	Salinity (o/oo)	Temperature (°C)	Salinity (o/oo)	Temperature (°C)	Salinity (o/oo)	Temperature (°C)	Salinity (o/oo)
1973 April	18.2 ± 3.4	23.1 ± 4.0	NS*	NS*	19.2 ± 2.5	6.5 ± 4.2	19.4 ± 2.1	25.3 ± 0.6
May	23.5 ± 0.9	30.4 ± 1.4	24.3 ± 0.8	24.6 ± 3.5	22.7 ± 0.6	13.5 ± 2.9	23.0 ± 1.3	27.2 ± 0.6
June	27.9 ± 0.1	33.8 ± 0.1	26.9 ± 1.0	16.6 ± 3.7	27.8 ± 0.6	11.0 ± 8.2	27.7 ± 0.6	28.0 ± 0.9
July	28.8 ± 0.7	28.8 ± 2.0	28.9 ± 0.5	28.1 ± 4.2	29.4 ± 0.5	11.1 ± 5.8	30.0 ± 0.5	28.2 ± 0.9
August	29.0 ± 0.5	30.8 ± 1.2	29.2 ± 0.8	30.9 ± 1.4	29.5 ± 0.6	18.9 ± 9.4	28.8 ± 0.8	29.4 ± 1.1
September	NS*	NS*	27.8 ± 0.4	29.8 ± 1.8	28.7 ± 0.3	22.2 ± 3.8	28.6 ± 0.9	29.9 ± 1.0
October	19.7 ± 0.4	34.7 ± 0.5	23.0 ± 4.7	28.8 ± 4.2	23.7 ± 3.6	23.1 ± 4.9	NS*	NS*
November	19.4 ± 0.6	34.3 ± 0.2	NS*	NS*	16.3 ± 0.2	23.0 ± 0.5	16.9 ± 0.2	32.1 ± 0.4
December	10.5 ± 0.3	34.2 ± 0.4	12.6 ± 0.2	30.9 ± 4.0	9.4 ± 0.4	19.8 ± 0.7	NS*	NS*
1974 January	14.9 ± 0.4	31.6 ± 0.8	15.6 ± 1.2	29.8 ± 2.0	14.0 ± 0.3	27.7 ± 2.6	13.7 ± 0.7	31.8 ± 0.1
February	12.5 ± 0.2	29.0 ± 1.4	12.8 ± 0.2	28.2 ± 3.5	16.1 ± 0.1	18.8 ± 6.0	15.9 ± 0.2	32.0 ± 0.3
March	15.1 ± 0.5	28.8 ± 1.0	16.0 ± 0.5	24.4 ± 2.9	17.1 ± 0.5	8.9 ± 5.9	16.2 ± 0.8	30.7 ± 0.1

*NS = No Sample

Table 2. Incidence of shell disease among pot-caught blue crabs, Callinectes sapidus, from field survey stations

Station: McClellanville (1)

	Males						Females								Grand Total	
	Juveniles		Adults		Total		Juveniles		Non-Ovig. Adults		Ovig. Adults		Total			
Month	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected
1973 April	15	0	190	4.2	205	3.9	35	2.9	6	0	0	0	41	2.4	246	3.7
May	23	0	266	7.9	289	7.3	55	0	0	0	1	100.0	56	1.8	345	6.4
June	12	8.3	67	9.0	79	8.9	23	0	3	0	0	0	26	0	105	6.7
July	28	3.6	197	5.6	225	5.3	25	0	14	0	0	0	39	0	264	4.6
August	19	5.3	180	4.4	199	4.5	20	0	22	0	0	0	42	0	241	3.7
September	NS*		NS*		NS*		NS*		NS*		NS*		NS*		NS*	
October	0	0	44	45.5	44	45.5	0	0	1	0	0	0	1	0	45	44.4
November	4	0	18	33.3	22	27.3	2	0	2	0	0	0	4	0	26	23.1
December	0	0	3	33.3	3	33.3	1	0	0	0	0	0	1	0	4	25.0
1974 January	0	0	13	15.4	13	15.4	1	0	5	20.0	0	0	6	16.7	19	15.8
February	6	0	16	25.0	22	18.2	4	0	0	0	0	0	4	0	26	15.4
March	10	10.0	39	17.9	49	16.3	14	7.1	0	0	0	0	14	7.1	63	14.3
Total	117	3.4	1,033	9.1	1,150	8.5	180	1.1	53	1.9	1	100.0	234	1.7	1,384	7.4

*NS = No Sample

Table 2. Incidence of shell disease among pot-caught blue crabs, Callinectes sapidus, from field survey stations (continued)

Station: Stono River (2)

Month	Males						Females								Grand Total	
	Juveniles		Adults		Total		Juveniles		Non-Ovig. Adults		Ovig. Adults		Total			
	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected
1973 April	NS*		NS*		NS*		NS*		NS*		NS*		NS*		NS*	
May	6	0	215	12.1	221	11.8	40	5.0	14	21.4	32	18.8	86	12.8	307	12.1
June	9	0	188	17.6	197	16.8	25	4.0	10	10.0	1	0	36	5.6	233	15.0
July	3	0	103	18.5	106	17.9	11	0	69	7.3	19	10.5	99	7.1	205	12.7
August	3	0	119	5.9	122	5.7	32	0	61	4.9	20	15.0	113	5.3	235	5.5
September	0	0	29	13.8	29	13.8	4	0	16	0	1	0	21	0	50	8.0
October	1	0	103	26.2	104	26.0	12	0	25	8.0	0	0	37	5.4	141	20.6
November	NS*		NS*		NS*		NS*		NS*		NS*		NS*		NS*	
December	4	0	40	37.5	44	34.1	14	0	1	0	0	0	15	0	59	25.4
1974 January	3	0	41	34.2	44	31.8	8	0	2	0	0	0	10	0	54	25.9
February	18	11.1	23	4.4	41	4.9	13	0	1	0	0	0	14	0	55	5.5
March	4	0	19	42.1	23	34.8	29	20.7	2	50.0	21	47.6	52	32.7	75	33.3
Total	51	3.9	880	17.5	931	16.8	188	4.8	201	7.5	94	22.3	483	9.3	1,414	14.2

*NS = No Sample

Table 2. Incidence of shell disease among pot-caught blue crabs, Callinectes sapidus, from field survey stations (continued)

Station: Ashepoo River (3)

	Males						Females								Grand Total	
	Juveniles		Adults		Total		Juveniles		Non-Ovig. Adults		Ovig. Adults		Total			
Month	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected
1973 April	18	0	130	3.1	148	2.7	30	0	71	0	0	0	101	0	249	1.6
May	2	0	94	6.4	96	6.3	29	0	5	0	0	0	34	0	130	4.6
June	5	20.0	226	8.9	231	9.1	21	0	17	23.5	0	0	38	10.5	269	9.3
July	8	0	147	2.7	155	2.6	29	3.4	11	0	0	0	40	2.5	195	2.6
August	5	0	115	10.4	120	10.0	42	0	23	0	0	0	65	0	185	6.5
September	0	0	17	11.8	17	11.8	4	0	17	0	0	0	21	0	38	5.3
October	0	0	93	10.8	93	10.8	14	7.1	66	4.6	0	0	80	5.0	173	8.1
November	0	0	76	19.7	76	19.7	2	0	3	0	0	0	5	0	81	18.5
December	6	0	23	0	29	0	9	0	1	0	0	0	10	0	39	0
1974 January	7	0	8	37.5	15	20.0	8	0	0	0	0	0	8	0	23	13.0
February	4	0	10	10.0	14	7.1	4	0	0	0	0	0	4	0	18	5.6
March	15	0	32	18.8	47	12.8	31	9.7	1	100.0	0	0	32	12.5	79	12.7
Total	70	1.4	971	8.5	1,041	8.1	223	2.2	215	3.7	0	0	438	3.0	1,479	6.6

*NS = No Sample

Table 2. Incidence of shell disease among pot-caught blue crabs, Callinectes sapidus, from field survey stations (continued)

Station: Beaufort River (4)

	Males						Females								Grand Total	
	Juveniles		Adults		Total		Juveniles		Non-Ovig. Adults		Ovig. Adults		Total			
Month	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected	No. Coll.	% Infected
1973 April	8	0	140	7.1	148	6.8	37	0	33	6.1	100	22.0	170	14.1	318	10.7
May	0	0	128	3.9	128	3.9	7	14.3	62	3.2	40	0	109	2.8	237	3.4
June	2	0	69	5.8	71	5.6	7	0	80	8.8	9	0	96	7.3	167	6.6
July	2	0	54	11.1	56	10.7	4	0	230	0.9	55	5.5	289	1.7	345	3.2
August	0	0	85	7.1	85	7.1	18	0	52	1.9	3	0	73	1.4	158	4.4
September	0	0	81	21.0	81	21.0	21	0	83	0	2	0	106	0	187	9.1
October	NS*		NS*		NS*		NS*		NS*		NS*		NS*		NS*	
November	0	0	44	56.8	44	56.8	0	0	5	20.0	0	0	5	20.0	49	53.1
December	NS*		NS*		NS*		NS*		NS*		NS*		NS*		NS*	
1974 January	3	0	23	39.1	26	34.6	1	0	0	0	0	0	1	0	27	33.3
February	7	0	14	7.1	21	4.8	1	0	1	0	0	0	2	0	23	4.4
March	2	0	14	42.9	16	37.5	16	6.3	3	66.7	2	100.0	21	23.8	37	29.7
Total	24	0	652	13.7	676	13.2	112	1.8	549	3.1	211	12.8	872	5.3	1,548	8.7

*NS = No Sample

Table 3. Total and monthly incidence (%) of shell disease among blue crabs from field survey stations

Month	Station				Total % Infected
	McClellanville	Stono	Ashepoo	Beaufort	
1973 April	3.7	NS*	1.6	10.7	5.8
May	6.4	12.1	4.6	3.4	7.2
June	6.7	15.0	9.3	6.6	10.1
July	4.6	12.7	2.6	3.2	5.4
August	3.7	5.5	6.5	4.4	5.0
September	NS*	8.0	5.3	9.1	8.4
October	44.4	20.6	8.1	NS*	17.5
November	23.1	NS*	18.5	53.1	30.1
December	25.0	25.4	0	NS*	15.7
1974 January	15.8	25.9	13.0	33.3	23.6
February	15.4	5.5	5.6	4.4	7.4
March	14.3	33.3	12.7	29.7	21.7
Overall % Infected	7.4	14.2	6.6	8.7	9.2

*NS = No Sample

Table 4. Incidence of shell disease by sex among blue crabs from field survey stations

	Males		
	No. Collected	No. Infected	% Infected
Juvenile	262	7	2.7
Adult	3,536	420	11.9
Total	3,798	427	11.2

	Females		
	No. Collected	No. Infected	% Infected
Juvenile	703	18	2.6
Non-ovigerous Adult	1,018	41	4.0
Ovigerous Adult	306	49	16.0
Total Adult	1,324	90	6.8
Grand Total	2,027	108	5.3

Table 5. Incidence of shell disease by sex and location among blue crabs in commercial catches from major South Carolina fishing grounds, January 1973 to February 1974

Location: North Edisto River

Sampling Period	Males		Females		Total	
	Sample Size	% Infected	Sample Size	% Infected	Sample Size	% Infected
1/01/73- 1/31/73	37	2.7	26	0.0	63	1.6
2/01/73- 2/28/73	33	0	67	1.5	100	1.0
3/01/73- 3/31/73	NS*		NS*		NS*	
4/01/73- 5/08/73	NS*		NS*		NS*	
5/09/73- 5/23/73	44	0	5	0	49	0
5/24/73- 6/06/73	43	4.7	6	16.7	49	6.1
6/07/73- 6/20/73	NS*		NS*		NS*	
6/21/73- 7/03/73	99	2.0	1	0	100	2.0
7/04/73- 7/18/73	NS*		NS*		NS*	
7/19/73- 8/01/73	NS*		NS*		NS*	
8/02/73- 8/15/73	48	4.2	2	0	50	4.0
8/16/73- 8/29/73	94	0	3	0	97	0
8/30/73- 9/12/73	36	0	9	0	45	0
9/13/73- 9/26/73	37	2.7	11	0	48	2.1
9/27/73-10/10/73	NS*		NS*		NS*	
10/11/73-10/24/73	NS*		NS*		NS*	
10/25/73-11/07/73	NS*		NS*		NS*	
11/08/73-11/21/73	NS*		NS*		NS*	
11/22/73-12/31/73	NS*		NS*		NS*	
1/01/74- 1/31/74	NS*		NS*		NS*	

*NS = No Sample

Table 5. Incidence of shell disease by sex and location among blue crabs in commercial catches from major South Carolina fishing grounds, January 1973 to February 1974 (continued)

Location: Port Royal Sound

Sampling Period	Males		Females		Total	
	Sample Size	% Infected	Sample Size	% Infected	Sample Size	% Infected
1/01/73- 1/31/73	34	2.9	159	5.0	193	4.7
2/01/73- 2/28/73	183	0.5	10	0.0	193	0.5
3/01/73- 3/31/73	53	1.9	33	0.0	86	1.2
4/01/73- 5/08/73	NS*		NS*		NS*	
5/09/73- 5/23/73	123	1.6	138	2.1	261	1.9
5/24/73- 6/06/73	NS*		NS*		NS*	
6/07/73- 6/20/73	98	3.1	5	0.0	103	2.9
6/21/73- 7/03/73	97	2.1	103	3.9	200	3.5
7/04/73- 7/18/73	82	4.9	117	0.0	199	2.0
7/19/73- 8/01/73	108	3.7	42	11.9	150	6.0
8/02/73- 8/15/73	170	1.7	24	0.0	194	1.5
8/16/73- 8/29/73	161	0.6	14	0.0	175	0.5
8/30/73- 9/12/73	46	0.0	1	0.0	47	0.0
9/13/73- 9/26/73	115	1.7	23	0.0	138	1.4
9/27/73-10/10/73	124	10.5	25	0.0	149	8.7
10/11/73-10/24/73	180	9.4	64	3.1	244	7.8
10/25/73-11/07/73	46	4.3	54	0.0	100	2.0
11/08/73-11/21/73	100	10.0	0	0.0	100	10.0
11/22/73-12/31/73	168	22.0	26	3.8	194	19.6
1/01/74- 1/31/74	36	25.0	112	0.9	148	6.8

*NS = No Sample

Table 5. Incidence of shell disease by sex and location among blue crabs in commercial catches from major South Carolina fishery grounds, January 1973 to February 1974 (continued)

Location: St. Helena Sound

Sampling Period	Males		Females		Total	
	Sample Size	% Infected	Sample Size	% Infected	Sample Size	% Infected
1/01/73- 1/31/73	184	0	51	3.9	235	0.9
2/01/73- 2/28/73	NS*		NS*		NS*	
3/01/73- 3/31/73	154	0	4	0	158	0
4/01/73- 5/08/73	NS*		NS*		NS*	
5/09/73- 5/23/73	216	1.4	99	4.0	315	2.2
5/24/73- 6/06/73	193	0	57	7.0	250	1.6
6/07/73- 6/20/73	565	2.3	71	7.0	636	2.8
6/21/73- 7/03/73	612	1.5	136	8.1	746	2.7
7/04/73- 7/18/73	571	3.0	34	2.9	605	3.0
7/19/73- 8/01/73	492	2.8	13	0	505	2.8
8/02/73- 8/15/73	480	4.0	7	28.6	487	4.3
8/16/73- 8/29/73	369	1.4	0	0	369	1.4
8/30/73- 9/12/73	178	0.6	5	0	183	0.5
9/13/73- 9/26/73	480	1.7	88	2.3	568	1.8
9/27/73-10/10/73	261	1.1	77	1.2	338	1.1
10/11/73-10/24/73	350	4.3	96	5.2	446	4.5
10/25/73-11/07/73	466	6.9	76	3.9	542	6.5
11/08/73-11/21/73	78	6.4	15	6.7	93	6.5
11/22/73-12/31/73	90	11.1	5	0	95	10.5
1/01/74- 1/31/74	57	7.0	35	8.6	92	7.6

*NS = No Sample

Table 5. Incidence of shell disease by sex and location among blue crabs in commercial catches from major South Carolina fishery grounds, January 1973 to February 1974 (continued)

Location: Calibogue Sound

Sampling Period	Males		Females		Total	
	Sample Size	% Infected	Sample Size	% Infected	Sample Size	% Infected
1/01/73- 1/31/73	3	0	70	1.4	73	1.4
2/01/73- 2/28/73	NS*		NS*		NS*	
3/01/73- 3/31/73	22	4.5	167	0	189	0.5
4/01/73- 5/08/73	NS*		NS*		NS*	
5/09/73- 5/23/73	NS*		NS*		NS*	
5/24/73- 6/06/73	NS*		NS*		NS*	
6/07/73- 6/20/73	14	0	36	2.8	50	2.0
6/21/73- 7/03/73	NS*		NS*		NS*	
7/04/73- 7/18/73	30	3.3	69	7.2	99	6.1
7/19/73- 8/01/73	83	6.0	16	6.3	99	6.1
8/02/73- 8/15/73	46	2.2	4	0	50	2.0
8/16/73- 8/29/73	144	3.5	48	2.1	192	3.1
8/30/73- 9/12/73	NS*		NS*		NS*	
9/13/73- 9/26/73	32	0	16	6.3	48	2.1
9/27/73-10/10/73	NS*		NS*		NS*	
10/11/73-10/24/73	NS*		NS*		NS*	
10/25/73-11/07/73	NS*		NS*		NS*	
11/08/73-11/21/73	NS*		NS*		NS*	
11/22/73-12/31/73	NS*		NS*		NS*	
1/01/74- 1/31/74	NS*		NS*		NS*	

*NS = No Sample

Table 6. Total incidence of shell disease by sex and location among blue crabs in commercial catches, January 1973 to February 1974

Location	Males			Females			Total		
	Sample Size	No. Infected	% Infected	Sample Size	No. Infected	% Infected	Sample Size	No. Infected	% Infected
North Edisto River	471	8	1.70	130	2	1.54	601	10	1.66
St. Helena Sound	5,796	158	2.73	869	44	5.06	6,665	202	3.03
Port Royal Sound	1,924	112	5.82	950	24	2.53	2,874	136	4.73
Calibogue Sound	374	13	3.48	426	10	2.35	800	23	2.88
Grand Total	8,565	291	3.40	2,375	80	3.37	10,940	371	3.39

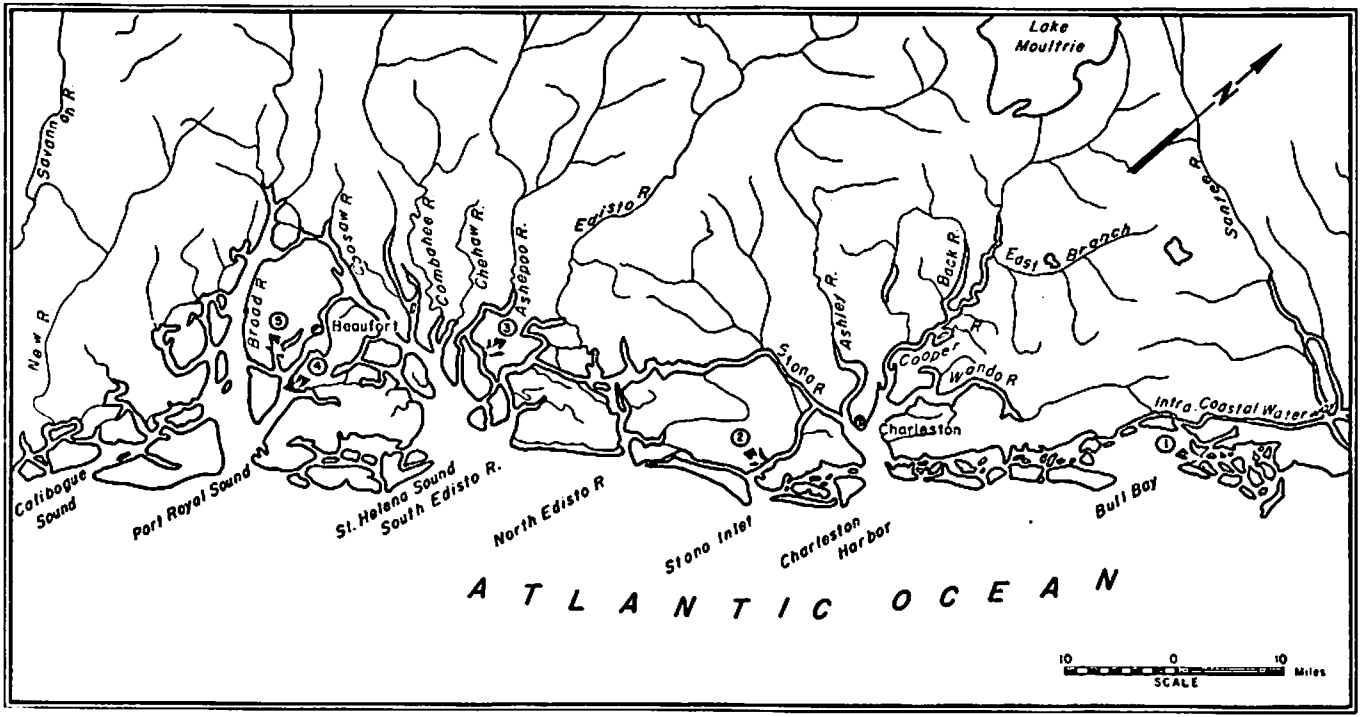

Figure 1. Blue crab sampling locations along the South Carolina coast: 1) McClellanville station, 2) Stono River station, 3) Ashepoo River station, 4) Beaufort River station, and 5) Blue Channel Corporation

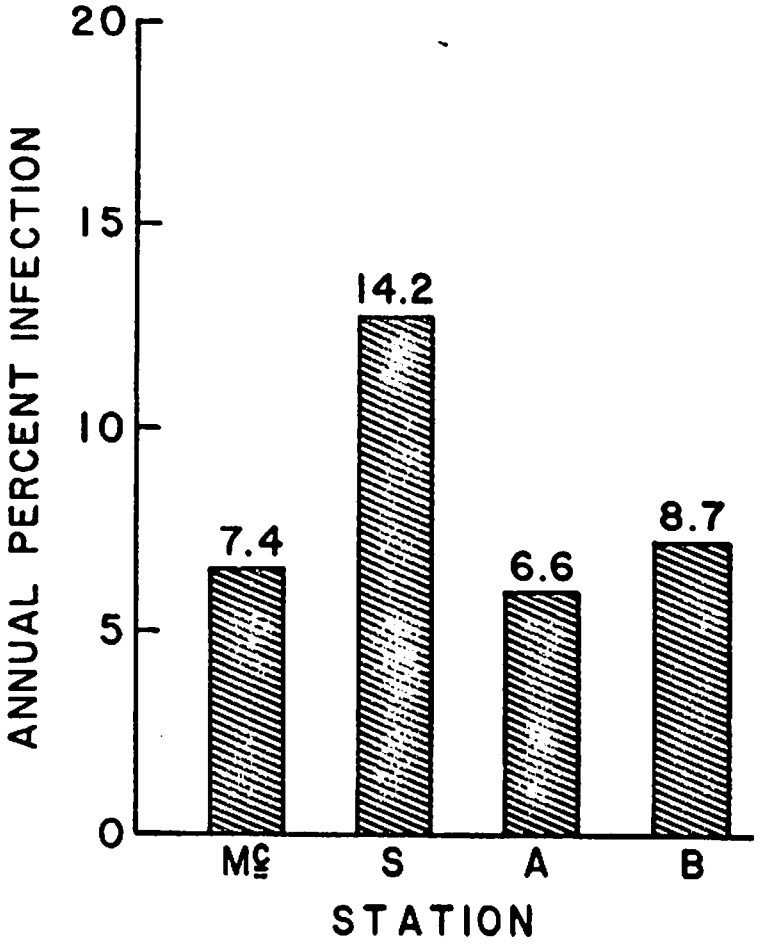

Figure 2. Annual incidence (%) of shell disease among blue crabs from field survey stations (Mc = McClellanville station, S = Stono River station, A = Ashepoo River station, and B = Beaufort River station)

STUDIES ON *Loxothylacus texanus*

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INTRODUCTION

The parasite, *Loxothylacus texanus* Boschma (1933) is a sacculinid parasite that infects immature blue crabs, *Callinectes sapidus* Rathbun, in the Gulf of Mexico. A free swimming cypris larva innoculates a young molting crab with cells that give rise to an endoparasitic root system. Following some subsequent molt, part of this root system breaks through to the outside as a sac on the hosts abdomen. This externa contains male and female gonads and a brood pouch. The parasitic barnacle is a self fertilizing hermaphrodite (Reinhard, 1950a).

Secondary sex characteristics of parasitized crabs are transformed to those of adult females. Gonadal development is suppressed, and once the externa emerges, molting and further growth ceases (Reinhard 1950a, 1950b, and 1956). Infected crabs are lost as fishery resources because they do not attain commercial size (130 mm in carapace width) and do not reproduce.

Our knowledge of the ecology and distribution of the barnacle is slight. Most papers (Christmas, 1969; Daugherty, 1952; Gunter, 1950; Park, 1969; Reinhard 1950a; and Wass, 1955) that mention incidence of infection are based on incidental observations and often on small sample size. The work of Atkins (1972) is more extensive in this regard.

The findings of Harris and Ragan (1970) suggest that the parasite may be important in the more saline bays of Louisiana but that lower salinities might be limiting to the organism. The objective of this study is to establish the incidence of Loxothylacus externae on Louisiana blue crabs from different salinity environments.

MATERIALS AND METHODS

Blue crabs were obtained during 1972 from Bayous Cypremort and Jean LaCroix and from Caminada Bay (Fig. 1). The crabs were captured with trawls and dip nets. All specimens were examined for externae of Loxothylacus texanus, and the stage of sac maturity was observed. Each crab was measured in carapace width to the nearest millimeter, and the sex of each crab was noted. Salinities and temperatures of water were recorded on each sampling date.

STUDY AREAS

Bayou Cypremort (Fig. 1) in south central Louisiana is a low salinity environment which ranged between one and eight parts per thousand and averaged three ppt. during the study. Bayou Jean LaCroix (Fig. 1) is a region of intermediate salinity (range = 4–20; average = 14 ppt) which is located in southeastern Louisiana about 15 miles southeast of Houma. Caminada Bay is an area of high salinities located in the Grand Isle region of southeastern Louisiana (Fig. 1). Values at this site varied between 19 and 28 ppt and averaged 24 ppt. Salinities and temperatures are shown for each area by month in Table 1. Salinities are plotted in Figure 2.

RESULTS

Crabs harboring externae of Loxothylacus texanus ranged from 33 to 78 mm in carapace width. At Bayou Cypremort, 303 of 617 crabs sampled were within the infected size range and none contained parasitic externae. A total of 1219 crabs were obtained from Bayou Jean LaCroix, and 876 fell within the infected size range. Thirty-seven percent of these had externae (Table 2). At Caminada Bay 514 of a total 814 crabs were of vulnerable size and 30 percent exhibited externae (Table 2). Monthly incidence of externae (Tables 2 and 3; Fig. 3) was highest in May at both Bayou Jean LaCroix and Caminada Bay.

The percentage of mature and spent externae is presented in Table 4 and in Figures 4 and 5. Their incidence was 69 percent on parasitized hosts from Caminada Bay as compared to 24 percent on those from Bayou Jean LaCroix. The highest percentage of mature sacs was observed in May and June in Caminada Bay, and a secondary peak occurred in October (Table 4 and Fig. 5). At Bayou Jean LaCroix one peak of ripe externae was encountered during October (Table 4 and Fig. 5). Most hosts (86%) contained a single sac but the intensity ranged as high as eight sacs.

The incidence of parasitic externae is presented in relation to host size in Table 5 and Figure 6. The highest incidence occurred in the 51-60 mm grouping.

The incidence of externae is shown in relation to sex of host in Table 6. Males were more frequently infected at Bayou Jean LaCroix, whereas the opposite was true at Caminada Bay. Overall, 35 percent of the females contained externae as opposed to 34 percent for males.

DISCUSSION

Crabs harboring parasitic externae of Loxothylacus texanus ranged between 33 to 78 mm in width. This agrees closely with the ranges found by Reinhard (1950a) and Atkins (1972). Incidence of the parasite is based only on those crabs that were within the infected size range because (1) only external parasitization was being observed and (2) it is easier to compare the magnitude of parasitism between areas when the size composition of hosts is relevant and coincident.

On this basis, infection of crabs was 37 percent at the intermediate salinity area (Bayou Jean LaCroix) which had a mean of 14 ppt. Infection was 30 percent at the high salinity location (Caminada Bay) where the mean was 24 ppt. No externae were encountered at the low salinity site (Bayou Cypremort) where salt concentrations averaged 3 ppt. Hence, salinity appears to be a factor that limits the distribution of L. texanus but only as certain minimal values are reached. These critical concentrations lie between those found at Bayous Cypremort and Jean LaCroix. By contrast the blue crab can osmoregulate in water that is virtually fresh (Gunter, 1950).

Most hosts (86%) contained a single externa but multiple infections ranged as high as eight sacs per host. The heaviest intensity reported previously was four sacs per host (Reinhard 1950a).

In order to relate the incidence of externae to the actual level of infection, it would be necessary to consider size composition of hosts. The parasite tends to emerge near the middle of the infected size range (Table 3, Fig. 6). The highest incidence of sacs was in the 51-60 mm grouping, but the absence of externae on smaller crabs does not indicate noninfection since many of these crabs harbor L. texanus as an endoparasite. Hence, it is suggested that crabs smaller than 51 mm be excluded in any attempt to project actual infection based on external parasitization.

Figure 5 shows that the incidence of externae on crabs was highest in May at Bayou Jean LaCroix as well as Caminada Bay. The desire to correlate this finding with temporal aspects in the life cycles of parasite and host is prohibited by the fact that the duration of the barnacle's endoparasitic stage is unknown. Reinhard (1951) maintains that L. texanus becomes external about eight to nine months after infection. He bases this on the assumption that the life cycle of this parasite is comparable to that of Sacculina carcini. However, there is reason to suspect that the period of emergence is shorter for L. texanus. Sacculinids do not normally inhibit molting until after the externa appears (Reinhard, 1956). If so in this case, internally parasitized blue crabs would obtain adult size before externae emerge, but this is not the case. On the other hand, it is quite possible that the endoparasitic stage of L. texanus does suppress blue crab growth and that the lengthy incubation period of S. carcini is applicable.

In defense of hypothesizing a shorter incubation for L. texanus, it is noted that the life cycle of S. carcini was studied in a colder water regime than is found in Louisiana coastal waters. In any case, there is a need to determine the interval between infection and emergence of L. texanus. This can only be accomplished by inducing infection of crabs in the laboratory.

There is yet another factor which complicates the problem of interpreting the seasonal incidence of externae on crabs. Samples may be dominated by infected crabs whenever normal juveniles are uncommon at sampling sites. The resulting increase in the percentage of externae may reveal more about recruitment of juvenile crabs than it does about fluctuations in the level of Loxothylacus infection. Darnell (1959) indicates that crabs within the infected size range are not abundant in Louisiana during May. Thus, light recruitment might have been at least partially responsible for the heavy infestation observed during this month.

The seasonal pattern of the incidence of mature and spent sacs is more informative and easier to interpret. Figure 4 illustrates that the percentage of ripe and spent sacs was much greater in the high salinity area. This is to be expected since spawning of the blue crab occurs in waters of high salinity

(Darnell, 1959; Churchill, 1919; and Van Engel, 1958). Completion of the parasite's life cycle depends on its ability to infect newly spawned young of the blue crab. This can be accomplished only if its spawning location corresponds to that of its single host.

It is likewise important that the parasite's spawning season be synchronous with that of its host. The work of Darnell (1959) suggests that female blue crabs move down the estuarine system to spawn in the spring and that there is a secondary spawning peak in the fall. Figure 5 indicates that the same is true for Loxothylacus. This trend is especially evident at the high salinity location (Caminada Bay) which is regarded as the more representative location. It appears that while some spawning may occur at Bayou Jean LaCroix, this activity is marginal, being influenced by salinity patterns. The fact that the spring spawning peak was smaller than the fall peak may be explained on this basis. Salinities during May (Table 1) were only four ppt as compared to 17-20 ppt during August and September when the highest percentage of mature sacs was recorded.

It appears that the migratory behavior of crabs parasitized by Loxothylacus corresponds to that of normal crabs specifically to normal females. Figure 3 demonstrates that despite the overall lower incidence of infested crabs in the high salinity area, the percentage was actually higher than that at the middle salinity region during peak spawning times. This may reflect movement of infested crabs down the estuarine system to the more saline reaches. Since the migration is necessary to the completion of its life cycle, does the parasite that physically feminizes the crabs also induce the migration that adult females make?

It may seem surprising that the overall incidence of L. texanus was lower at the high salinity region where most of the spawning occurs. This too can be explained on the basis of typical crab migration. Darnell (1959) states, "Young crabs migrate from these outside waters to the inside fresher areas such as Lake Pontchartrain. Here the smallest crabs tend to seek out the freshest and shallowest marginal areas for feeding and growth." The results of this investigation suggest that the abnormal crabs are migrating in the same manner as the normal ones.

Reinhard (1956) states that sacculinids tend to infect males more frequently than females. This was not found to be the case in the present study. At Caminada Bay 28 percent of the males had externae as compared to 32 percent of the females. Males were more heavily infected (39 to 36 percent) at Bayou Jean LaCroix. By combining the actual numbers for both areas, infestations of the two sexes were found to be virtually equal (Table 6).

Overall, females far outnumbered males in both areas (Table 6). These ratios are suspect, however, because many apparently normal females may have been males in some stage of endoparasitic modification.

The total absence of externae on crabs from the low salinity location would appear surprising unless low salinity kills the endoparasite or arrests its development to the extent that it does not become external. One of these explanations must hold as the crabs were spawned in more saline waters and would have been subjected to infection at some time early in life.

The absence of parasitic externae in crabs from Bayou Cypremort has prompted laboratory holding experiments now in progress. Preliminary laboratory work has indicated that L. texanus does not emerge on crabs in a salinity of three ppt (the mean for Bayou Cypremort) but that it does become external on hosts in the salinities found in the other two locations. Preliminary work has also indicated that crabs with mature sacs die when placed in the lower salinity. This occurs after their externae embibe and subsequently rupture. This abnormal development was not observed in crabs from the same population that were placed in the higher salinities.

The present field work has demonstrated that the parasite L. texanus is a common parasite of the blue crab in the more saline bays of coastal Louisiana but that lower salinities appear to limit its distribution. Thus, the fresher bays may be more important in terms of crab production. The parasite should be taken into account in any future attempt to manage the crab fishery in Louisiana. Christmas (1969) has recognized the potential threat that L. texanus poses to the Gulf coast crab fishery.

These findings may also have valuable application in mariculture. It would certainly be advisable to consider the salinity limitations of such an important parasite when selecting a site for crab farming. This knowledge may also be applicable to shrimp farming where some investigators have found that adult crabs are serious predators that are difficult to control. The potential use of L. texanus as a biological control in shrimp and possibly other extensive maricultural operations warrents serious consideration.

LITERATURE CITED

Atkins, Gerald. 1972. Notes on the occurrence and distribution of the rhizocephalan parasite (Loxothylacus texanus Boschma) of blue crabs (Callinectes sapidus Rathbun) in Louisiana estuaries. La. Wildl. and Fish. Comm. Tech. Bull. No. 2. 13 p.

Boschma, H. 1933. New species of Sacculinidae in the collection of the United States National Museum. Tindschr. ned. dierk. Ver Leiden 3(3):219-41.

Boschma, H. 1933. New species of Sacculinidae in the collection United States National Museum. Tindschr. ned. dierk. Ver Leiden 3(3):219-41.

Christmas, J. Y. 1969. Parasitic barnacles in Mississippi estuaries with special reference to Loxothylacus texanus Boschma in the blue crab (Callinectes sapidus). Proc. 22nd Annu. Conf., Southeast Assoc. Game and Fish. Comm. 272-5.

Churchill, E. P. 1919. Life history of the blue crab. U. S. Bur. Fish, Bull. (1917-18), Vol. 36 (870):95-128.

Darnell, R. M. 1959. Studies of the life history of the blue crab (Callinectes sapidus Rathbun) in Louisiana waters. Trans. Amer. Fish. Soc., 88(4):294-04.

Daugherty, F. M., Jr. 1952. The blue crab investigation, 1949-1950. Texas Jour. Sci. 1:70-84.

Gunter, Gordon. 1950. Seasonal population changes and distributions as related to salinity, of certain invertebrates of the Texas Coast, including the commercial shrimp. Publ. Inst. Mar. Sci. Univ. Texas. 1(2):7-51.

Harris, A. H., and J. G. Ragan. 1970. Observations on the ecology and incidence of Loxothylacus texanus (Boschma), parasitic in the blue crab (Callinectes sapidus Rathbun) in south Louisiana. Proceeding of the Southwestern Assoc. of Parasit. Abstract.

Park, John R. 1969. Preliminary study of Biscayne Bay. Quart. J. Fla. Acad. Sci, Vol 32, No. 1, p. 12-20.

Reinhard, E. G. 1950a. An anlysis of the effect of a sacculinid parasite on the external morphology of Callinectes sapidus Rathbun. Biol. Bull. 98(3):277-88.

Reinhard, E. G. 1950b. The morphology of Loxothylacus texanus of the blue crab. Tex. Jour. Sci., (3):360-5.

Reinhard, E. G. 1951. Loxothylacus, a parasite of the blue crab in Texas. Texas Game and Fish., April, 1951, pp 14-7.

Reinhard, E. G. 1956. Parasitic castration of crustacea. Parasitology 5:79-107.

Van Engel, W. A. 1958. The blue crab and its fishery in Chesapeake Bay. Part 1 Reproduction and early development, growth, and migration. U.S. F & W. S., Comm. Fish. Rev., Vol. 20(6):6-17.

Wass, M. L. 1955. Decapod crustaceans of Alligator Harbor. Quart. J. Fla. Acad. Sci., Vol. 18, No. 3, p. 129-76.

Table 1. Temperatures and salinities of Bayou Cypremort, Bayou Jean LaCroix, and Caminada Bay during 1972

Month	Bayou Cypremort		Bayou Jean LaCroix		Caminada Bay	
	Temp (C)	Salinity (ppt)	Temp (C)	Salinity (ppt)	Temp (C)	Salinity (ppt)
Jan.	14	1	19	11	17	19
Feb.	11	1	23	9	21	25
March	18	1	24	10	25	22
April	24	1	27	16	26	20
May	27	2	25	4	27	26
June	33	1	32	16	33	25
July	34	2	34	14	34	28
Aug.	34	8	31	17	31	25
Sept.	31	6	32	20	27	26
Oct.	26	6	26	19	-	-
Nov.	22	6	22	16	-	-
Dec.	-	-	-	-	-	-

Table 2. Incidence of externae on crabs by sampling month at Bayou Jean LaCroix

Month	Total No. Crabs	Crabs In Infected Range	Crabs With Externae	Percent With Externae	No. Externae Per Host	Range Of Intensity
Jan.	222	170	54	32	1.5	1-8
Feb.	117	93	17	18	1.6	1-5
Mar.	110	80	26	33	1.3	1-4
April	115	84	26	31	1.2	1-5
May	143	118	73	62	1.3	1-3
June	81	57	35	61	1.3	1-3
July	79	46	23	50	1.2	1-2
Aug.	102	25	2	8	1	1-1
Sept	72	42	12	29	1.3	1-2
Oct.	83	75	39	52	1.1	1-3
Nov.	95	86	17	20	1.2	1-2
Dec.	-	-	-	-	-	-
Total/ Average	1219	876	324	37	1.3	1-8

Table 3. Incidence of externae on crabs by sampling month at Caminada Bay

Month	Total No. Crabs	Crabs In Infected Range	Crabs With Externae	Percent With Externae	No. Externae Per Host	Range Of Intensity
Jan.	-	-	-	-	-	-
Feb.	27	6	3	50	1.0	0-1
Mar.	79	59	6	10	1.2	0-2
April	139	101	21	21	1.2	0-3
May	145	95	60	63	1.3	0-3
June	50	29	3	10	1.0	0-1
July	77	32	9	28	1.7	0-5
Aug.	132	81	21	27	1.3	0-4
Sept.	76	23	2	9	1.0	0-1
Oct.	109	88	31	35	1.4	0-3
Nov.	-	-	-	-	-	-
Dec.	-	-	-	-	-	-
Total/ Average	834	514	156	30	1.2	0-5

Table 4. Percentage of crabs with immature, developing, and mature or spent externae at Bayou Jean LaCroix and Caminada Bay during 1972

Month	Immature Externae		Developing Externae		Mature or Spent Externae	
	Jean LaCroix	Caminada	Jean LaCroix	Caminada	Jean LaCroix	Caminada
Jan.	100	-	0	-	0	-
Feb.	100	100	0	0	0	0
Mar.	100	33	0	0	0	67
April	85	14	15	10	0	76
May	31	0	43	0	26	100
June	75	0	25	0	0	100
July	96	22	0	0	4	78
Aug.	23	68	22	0	50	32
Sept.	42	100	8	0	50	0
Oct.	80	0	10	35	10	35
Nov.	18	-	-	-	82	-
Dec.	-	-	-	-	-	-

Table 5. The incidence of parasitic externae in relation to host size at Bayou Jean LaCroix and Caminada Bay

Carapace Width (mm)	No. Sampled	No. With Externae	Percent With Externae
	Bayou Jean LaCroix		
31-40	292	41	14
41-50	274	125	46
51-60	196	128	65
61-70	70	27	39
71-80	44	3	7
	Caminada Bay		
31-40	105	11	11
41-50	112	48	43
51-60	106	60	57
61-70	101	32	32
71-80	90	5	6
	Combined		
31-40	397	52	13
41-50	386	173	45
51-60	302	188	62
61-70	171	59	35
71-80	134	8	6

Table 6. Relationship between incidence of externae and host sex at Bayou Jean LaCroix and Caminada Bay

Area	Number Males		Number Females	
	Total	With Externae	Total	With Externae
Bayou Jean LaCroix	230	89 (39%)	646	235 (36%)
Caminada Bay	194	55 (28%)	320	101 (32%)
Combined	424	144 (34%)	966	336 (35%)

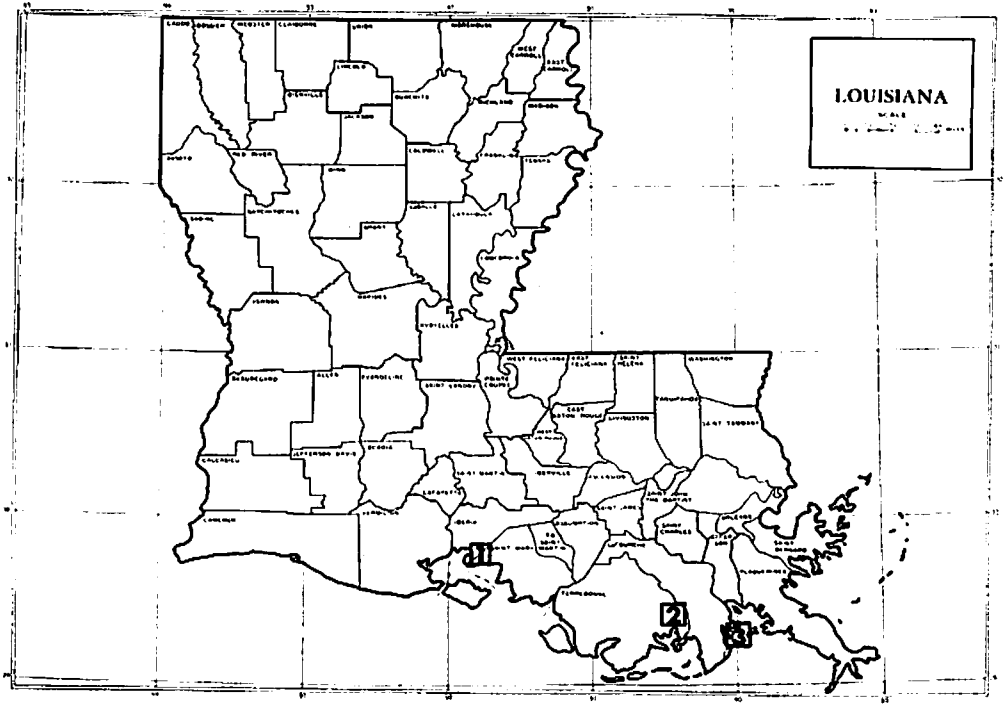

Figure 1. Map of Louisiana showing location of the three sampling sites. 1) Bayou Cypremort 2) Bayou Jean LaCroix 3) Caminada Bay.

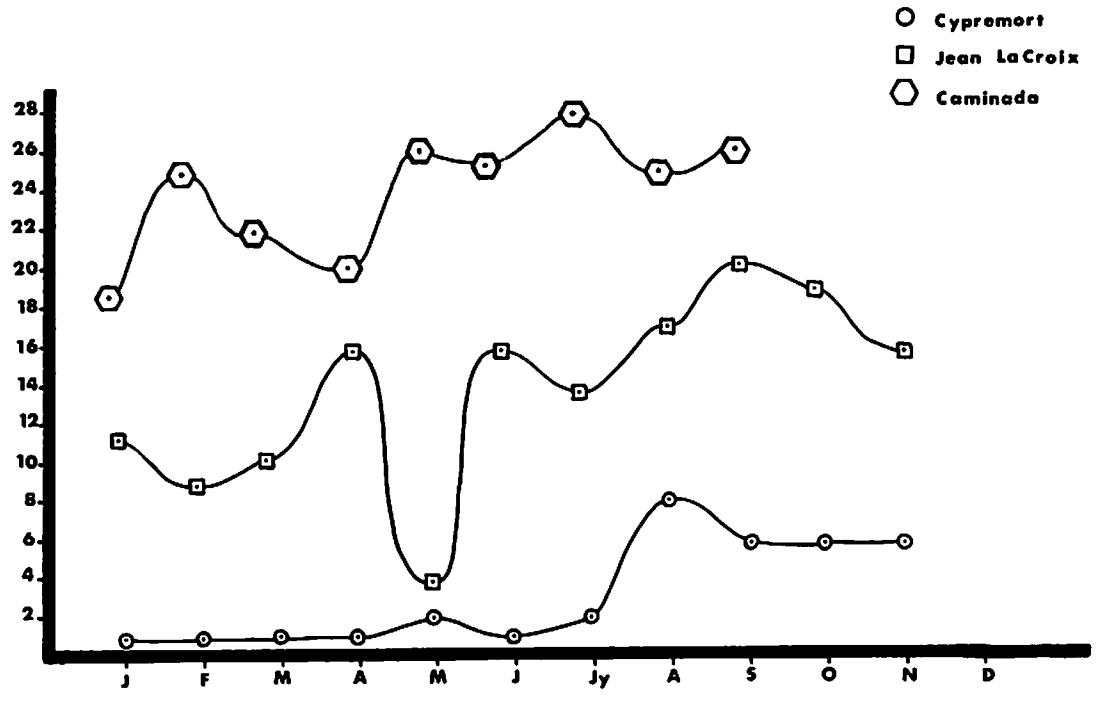

Figure 2. Salinities at Bayou Cypremort, Bayou Jean LaCroix, and Caminada Bay during 1972 (ppt).

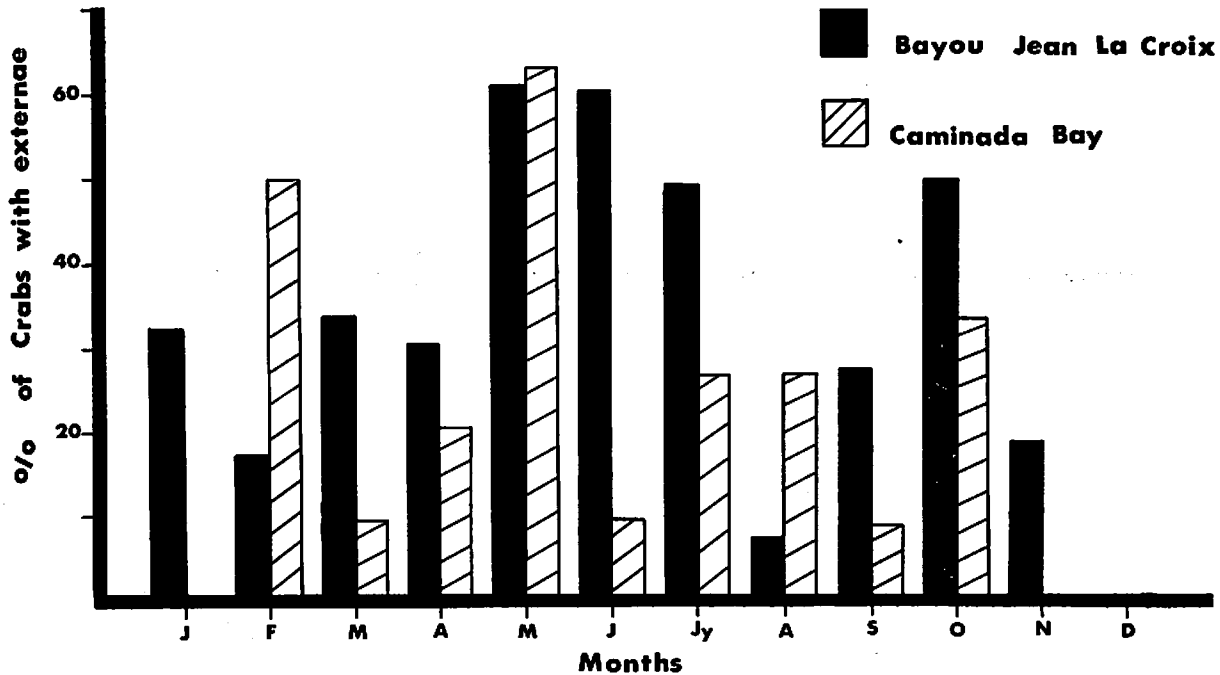

Figure 3. Incidence of Loxothylacus texanus in blue crabs from Bayou Jean Lacroix and Caminada Bay during 1972.

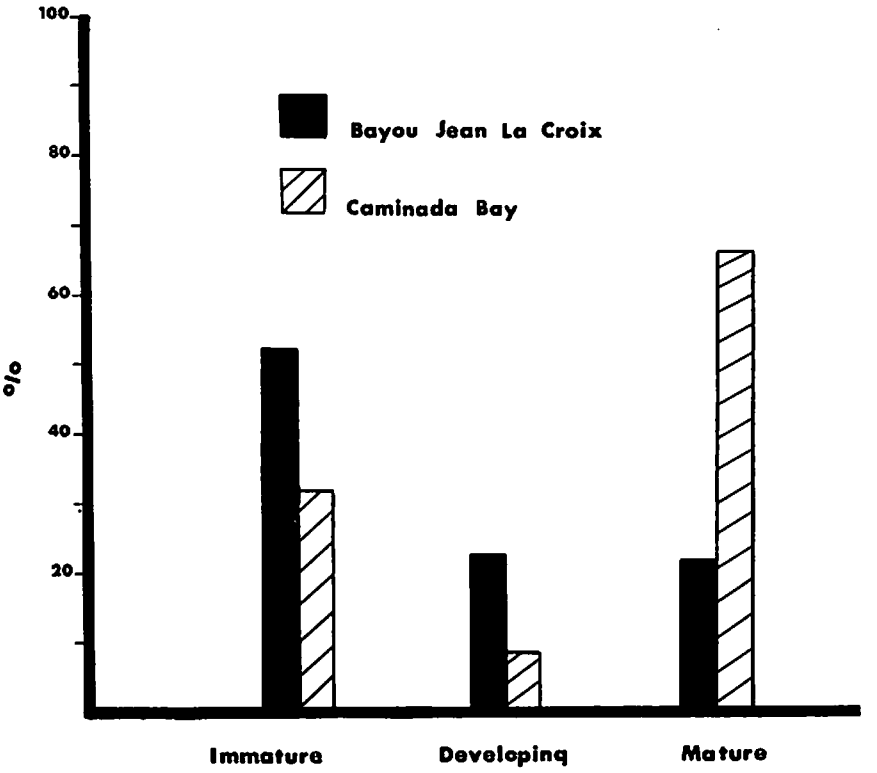

Figure 4. Sexual development of parasitic externae on crabs from Bayou Jean LaCroix and Caminada Bay during 1974.

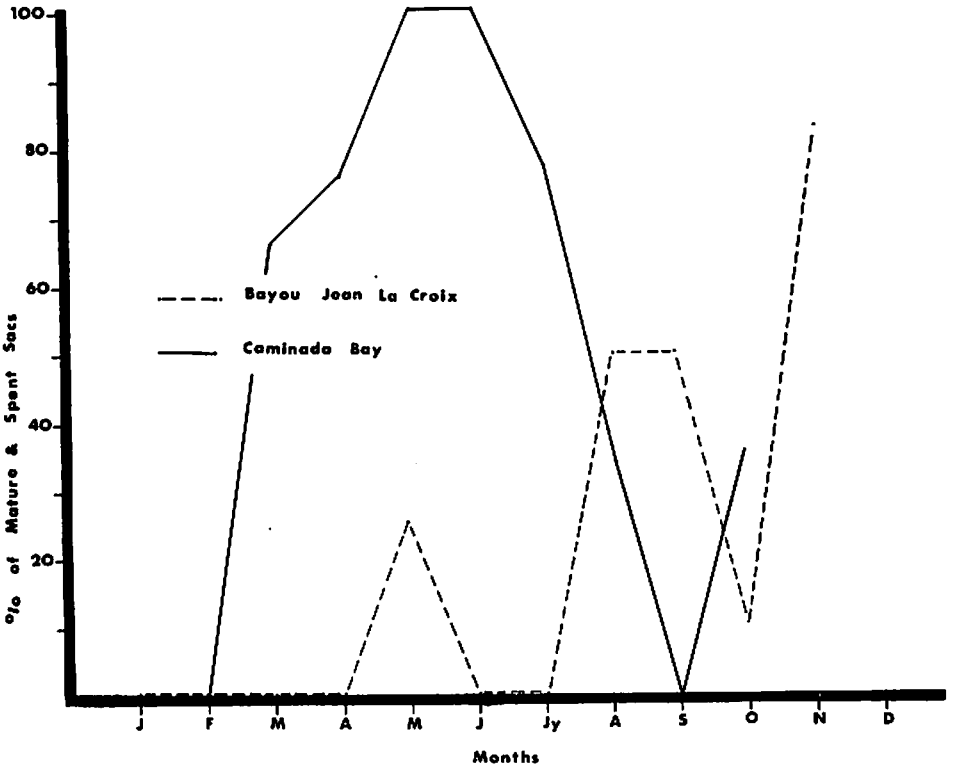

Figure 5. Seasonal incidence of mature and spent sacs from Bayou Jean LaCroix and Caminada Bay during 1972.

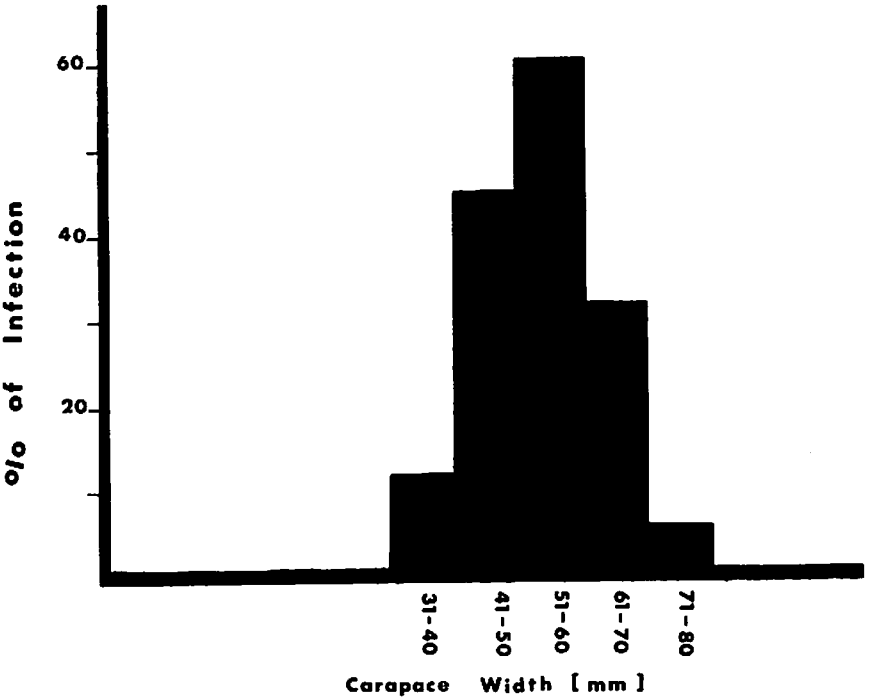

Figure 6. Size composition of crabs containing externae of Loxothylacus texanus

THE USE OF ANTIBIOTICS AND DIMETHYL-SULFOXIDE IN THE TREATMENT OF DISEASED BULLFROG TADPOLES, *Rana catesbeiana*[1]

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INTRODUCTION

In the years 1971-1974, septicemic frog disease severely reduced pond and laboratory populations of the Louisiana bullfrog (*Rana catesbeiana*). Bacteriological analyses of septicemic frogs in other laboratories have shown that *Aeromonas hydrophila*, *Aeromonas punctata*, *Alkaligenes faecalis*, *Pseudomonas fluorescens*, *Mima polymorpha*, *Staphylococcus epidermidis*, and *Citrobacter freundii* could be isolated from diseased frogs in individual epidemics (Miles, 1950; Reicherbach-Klinke and Elkan, 1965; Gibbs et al., 1966; Glorioso et al., 1974). Although it was suggested that *Aeromonas hydrophila* and *Mima polymorpha* were the major pathogens of Midwestern frogs (Gibbs et al., 1966), only a low incidence of *Aeromonas hydrophila* infections were observed among septicemic Louisiana bullfrogs (Glorioso et al., 1974). *Mima polymorpha* and a *Flavobacterium* sp. were the major pathogens isolated from the blood of diseased Louisiana bullfrogs, and a *Corynebacterium* sp. was observed in tissue abscesses. Furthermore, diseased tadpoles showed a high incidence of *Proteus* sp. infections.

A successful technique for the treatment of diseased frogs was reported by Gibbs (1963) and Gibbs et al. (1966). This

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treatment involved giving each frog 5 mg of tetracycline for each 30 g of body weight twice daily for 5 days by stomach tube. However, Amborski and Glorioso (1974) reported that some bacterial pathogens of frogs were resistant to tetracycline. Furthermore, the method reported by Gibbs proved to be combersome and not applicable to the treatment of diseased tadpoles. Since 70 percent of the mortalities observed in our laboratory occurred among metamorphosing animals, it became evident that a more practical method of treatment for diseased tadpoles had to be developed. This report presents the results of our search for an effective method of treatment for diseased tadpoles.

MATERIALS AND METHODS

Animals

Bullfrog tadpoles (Rana catesbeiana) were collected from aquatic sources in Central and South Louisiana. Tadpoles showing clinical signs of bacterial septicemia were separated from healthy tadpoles. Both healthy and diseased tadpoles were fed Purina minnowmeal, held in 189-liter polyvinyl plastic containers and clinical observations were made routinely.

Enumeration of Bacteria

Samples of maintenance water or homogenized tadpoles were serially diluted in sterile distilled water, plated on nutrient agar and incubated at 25 C. Total bacterial numbers were determined by the standard plate count method.

Antibiotic Sensitivity Tests

Antibiotic sensitivity discs (Baltimore Biological Laboratories) were used to determine which antibiotic(s) would inhibit the growth of bacteria shown to be pathogenic for tadpoles and adult bullfrogs (Glorioso et al., 1974). The antibiotics tested were tetracycline, sulfathiazole, triple-sulfa, terramycin, aureomycin, streptomycin, chloramphenical, and penicillin.

Treatment

Experiments were devised to evaluate the effect of dimethylsulfoxide (DMSO) and/or antibiotics on the viability of the test animals as well as on the bacterial numbers within the tissues and maintenance water of the test animals. Unless otherwise indicated, solutions of 1 percent DMSO and/or 100 µg/ml of each antibiotic were used as maintenance water. All experimental polyvinyl

plastic containers initially housed 35 nonfed tadpoles in 4 liters of maintenance solution. The containers were cleaned daily and the experimental conditions reinstated. Daily bacterial counts were made on 5 homogenized diseased tadpoles from each experimental condition. Tadpoles were homogenized by a Sorvall Omnimixer in 45 ml of sterile distilled water.

Animal Health

Animal health was evaluated on the basis of gross pathology. The presence of dermal abscesses with edema and hemorrhaging, hemorrhagic liver, hemorrhagic kidney, hemorrhagic and enlarged spleen, and intestinal wall hemorrhaging were each scored as one, and a cumulative point value was determined for each animal. No gross pathology was regarded as a zero score.

Statistical Analyses

The data were analysed using analysis of variance. Completely randomized designs were used in all experiments, incorporating factorial arrangements of treatments when applicable. To satisfy the assumption of homogeneity of variances, square root transformations of the data were used.

RESULTS

A combination of tetracycline and sufathizaole was found to effectively inhibit the growth of all bacterial pathogens tested. This antibiotic combination was then used in treating diseased tadpoles by introducing 100 µg/ml of each antibiotic into the maintenance water. Figure 1 shows the results of this treatment. Within 10 days all untreated animals were lost while only 34 percent of the antibiotic treated animals succumbed to bacterial disease. The majority of deaths in the treated group occurred within the first 24 hours of treatment presumably before the antibiotics could affect the bacteria.

In an attempt to increase the efficiency of the antibiotic treatment, dimethyl sulfoxide (DMSO), an agent demonstrated to increase the transport of materials into cells and animals, was introduced into the test system.

The toxicity of DMSO for tadpoles was determined by placing healthy tadpoles into containers with 20, 4, and 1 percent DMSO for 24 hours. Duplicate containers had antibiotics in addition to the DMSO. Table 1 shows the percent losses in viability resulting from these treatments. Although concentrations of DMSO above 1 percent proved to be toxic, solutions of 1 percent DMSO alone or with antibiotics had no apparent ill effects on healthy

tadpoles. Thus solutions of 1 percent DMSO and/or antibiotics were used in treating diseased tadpoles.

The decrease in tadpole mortality resulting from antibiotic treatment alone as shown in Figure 1 may have been due to the effect of the treatment on the bacteria within the maintenance water or on bacteria within the tadpole itself. Therefore, the changes in total bacterial numbers within the treated maintenance water were evaluated. Diseased tadpoles were placed in solutions of DMSO and/or antibiotics under both fed and nonfed conditions.

In addition, the changes in total bacterial numbers in flowing water (600 ml/min) were determined for both fed and nonfed tadpoles. Figure 2 shows the results of these experiments. The results indicate that under both fed and nonfed conditions, flowing water was significantly ($P<0.05$) more effective in reducing bacterial numbers in the water than DMSO antibiotic treated nonflowing water. Bacterial numbers were significantly ($P<0.05$) lower after 48 hours under treated or untreated nonfed conditions when compared with fed conditions. Under both fed and nonfed static water conditions DMSO significantly ($P<0.05$) increased the bacterial numbers after 48 hours. Overall, the presence of DMSO and/or antibiotics in the water during the first 24 hours had no significant effect on bacterial numbers in the water.

Since DMSO and/or antibiotic treatment did not significantly reduce the number of bacteria in the maintenance water the effects of the treatment shown in Figure 1 were surmised to have an effect on bacteria within the diseased animal. Figure 3 shows the changes in bacterial numbers within DMSO and/or antibiotic treated tadpoles over a 4 day period. During the first day, all conditions, except DMSO treated tadpoles, showed a significant ($P<0.01$) decline in bacterial numbers within the whole animal. At the end of 4 days, treated tadpoles had significantly ($P<0.05$) lower numbers of bacteria than untreated animals.

The decline in bacterial numbers within the whole animal may have resulted from a reduction in intestinal flora due to combinations of treatment, starvation and excretion. However, bacterial pathogens which reside within the tissues and contribute to the septicemic condition may not have been affected. Therefore changes in total bacterial numbers were evaluated in deceased tadpoles in which the lower intestinal tract had been surgically removed. These groups of animals were subjected to treatment solutions of 3 percent DMSO plus 1000 µg/ml of each antibiotic and to 1 percent DMSO and/or antibiotics at 100 µg/ml.

Figure 4 shows the changes in bacterial numbers over a 7 day period. Overall, the bacterial counts within the whole tadpole were initially 4-6 times greater than in animals in which the lower intestinal tract had been removed. Apparently, the major

losses in bacterial numbers in the whole animal were due to losses in intestinal bacterial numbers.

With the exception of the 48 hour sampling time tadpoles treated with 3 percent DMSO plus 1000 µg/ml of each antibiotic had significantly ($P<0.01$) greater numbers of bacteria per gram of tissue than all other treatments. Fourteen animals died during the treatment period and were replaced by 9 additional diseased tadpoles. The replacement of diseased tadpoles for dead ones likely accounts for the decrease in numbers at 48 hours. Figure 5 indicates that averaged across time all other treatments resulted in significantly ($P<0.01$) fewer numbers of bacteria per gram of tissue.

After 24 hours tadpoles treated with antibiotics (100 µg/ml each) alone or with 1 percent DMSO plus antibiotics (100 µg/ml each) had significantly ($P<0.05$) lower numbers of tissue bacteria than did untreated tadpoles. Averaged across time tadpoles treated with 1 percent DMSO plus antibiotics had significantly ($P<0.05$) fewer numbers of tissue bacteria than any other group tested; however, this difference was not evident after 7 days of treatment.

Figure 6 shows the changes in animal health over the 7 day period. At the end of 7 days of treatment, tadpole health was significantly ($P<0.05$) improved in all cases except those which were treated with 3 percent DMSO and antibiotics. The most dramatic improvement in health was demonstrated by tadpoles treated with antibiotics alone. Although the combined DMSO and antibiotic treatment lowered tissue numbers of bacteria more than other treatments, the organs of these animals showed more gross pathology and were unusually soft and lacked structural integrity.

DISCUSSION

Antibiotics administered by stomach tube have been successfully used in other laboratories in the treatment of adult frogs (Gibbs et al., 1966). However, this method is impractical for treating large numbers of diseased animals. Furthermore, since bacterial septicemia of Louisiana bullfrogs was especially fatal to bullfrog tadpoles, the need for improved methods of treatment became evident.

Before antibiotic treatments can be successfully carried out antibiotic sensitivity studies should be performed to insure that clinically important bacteria will be attacked within the animal. Although tetracycline and sulfathiazole proved to be an effective combination in inhibiting the growth of all bacteria previously shown to be pathogenic for the Louisiana bullfrog (Glorioso et al., 1974), it should be emphasize that the antibiotic(s) of choice may vary from epidemic to epidemic.

The results indicate that after 24 hours the bacterial numbers in flowing water containers are significantly lower than in static water containers despite treatment with DMSO and/or antibiotics. Since tadpoles can be infected by free living pathogens which can be found in the aquatic environment the isolation of healthy tadpoles in flowing water containers to insure low numbers of environmental pathogens would be an initial disease prevention measure.

Tadpoles showing clinical signs of bacterial septicemia should be detected early and isolated in static water containers. The containers should be cleaned and treated with 100 µg/ml of each antibiotic daily. This would prevent the accumulation of bacteria, expose the animals to the antibiotic therapy and reduce the expense one would encounter in a flowing water system. This method is supported by the reduction in diseased tadpole mortality among antibiotic treated populations. After 24 hours of treatment, antibiotics had no effect on environmental bacterial numbers, although they did significantly reduce bacterial counts within both the intestinal tract and the tissues of the test animals. In addition, the overall health of the antibiotic treated tadpoles was more rapidly improved than under non-treated conditions. The majority of deaths among both the antibiotic treated and untreated tadpoles occurred during the first 24 hours, and the untreated animals continued to show mortalities throughout the observation period.

Feeding diseased tadpoles during treatment is undesirable since the increased amount of organic material in the water supports the rapid growth of free living pathogens despite the presence of antibiotics. Moreover, the starvation of larval animals in the latter stages of development increases the rate of metamorphosis and apparently does not create starvation stress.

The use of DMSO is contraindicated because it proved to be either harmful to the animal or did not increase the overall effectiveness of the antibiotic treatment. The general health of tadpoles treated with DMSO appeared to be worse than untreated or antibiotic treated tadpoles. In addition, DMSO has been shown to concentrate in the thyroid gland of tadpoles and inhibit thyroxine production, a hormone essential to metamorphosis (Hammerman and Ritterman, 1969).

Our experience with bacterial disease among tadpoles has shown that simple precautions such as clean containers, flowing water, and proper diets can significantly reduce outbreaks of epidemics among crowded populations. However, despite these precautions, if members of a population begin showing clinical signs, they must be separated from healthy animals and treated immediately.

LITERATURE CITED

Amborski, R. L., and J. C. Glorioso. 1973. The frog revisited. Science 181:495.

Gibbs, E. L. 1963. An effective treatment for red-leg disease in Rana pipiens. Lab. Animal Care 13:781-3.

Gibbs, E. L., T. J. Gibbs, and P. C. Van Dyck. 1966. Rana pipiens: health and disease. Lab. Animal Care 16:142-60.

Glorioso, J. C., R. L. Amborski, J. M. Larkin, G. F. Amborski, and D. D. Culley. 1974. Laboratory identification of bacterial pathogens of aquatic animals. Am. J. Vet. Res. 35:447-50.

Hammerman, D. L., and D. P. R. Ritterman. 1969. Dimethylsulfoxide: influence upon frog tadpole metamorphosis. J. Exp. Physiology 54:223-8.

Miles, E. M. 1950. Red-leg in tree frogs caused by Bacterium alkaligenes. J. Gen. Microbiol. 4:434-6.

Reichenbach-Klinke, H., and E. Elkan. 1965. The principal diseases of lower vertebrates. Academic Press. New York.

Table 1. The effects of DMSO and antibiotics on the viability of healthy tadpoles. Given in terms of percent viability.

Treatment						
20% DMSO	20% DMSO & antibio.	4% DMSO	4% DMSO & antibio.	1% DMSO	1% DMSO & antibio.	water
0	0	30	20	100	100	97

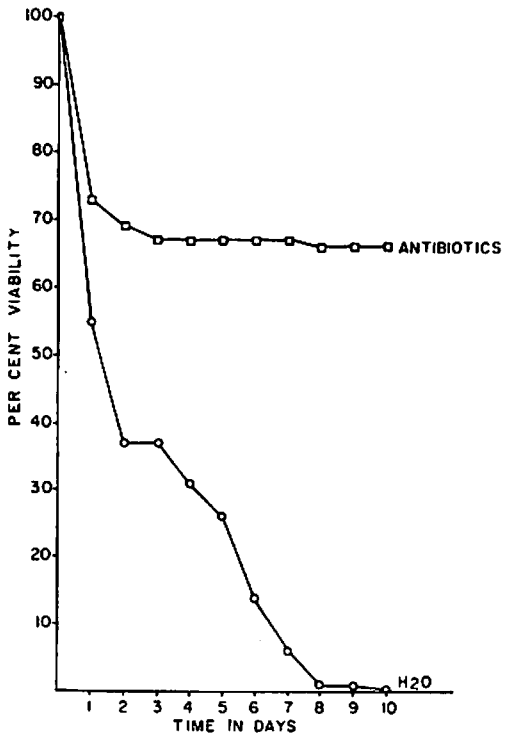

Figure 1. A comparison of the percent loss in viability among antibiotic treated and nontreated diseased bullfrog tadpoles.

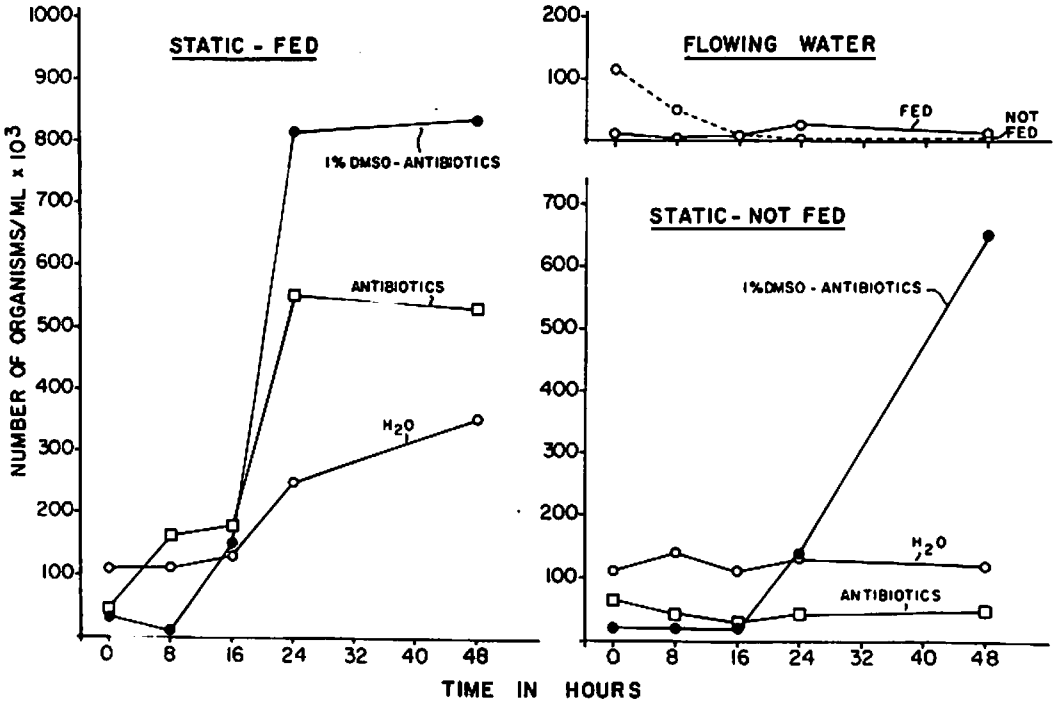

Figure 2. The change in total bacterial counts within both static and flowing maintenance water containing combinations of food, DMSO, and antibiotics.

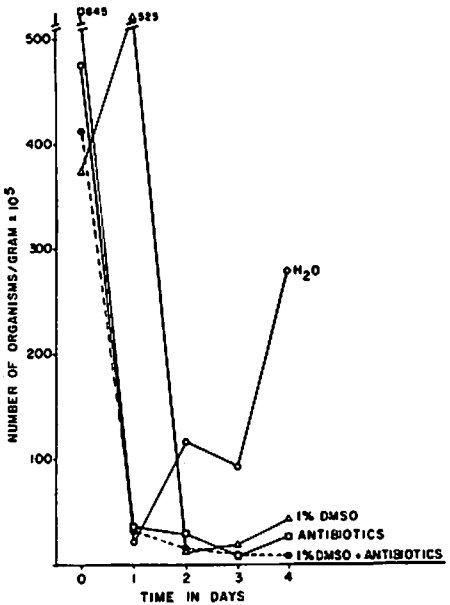

Figure 3. The change in total bacterial counts within homogenized diseased bullfrog tadpoles treated with DMSO and/or antibiotics.

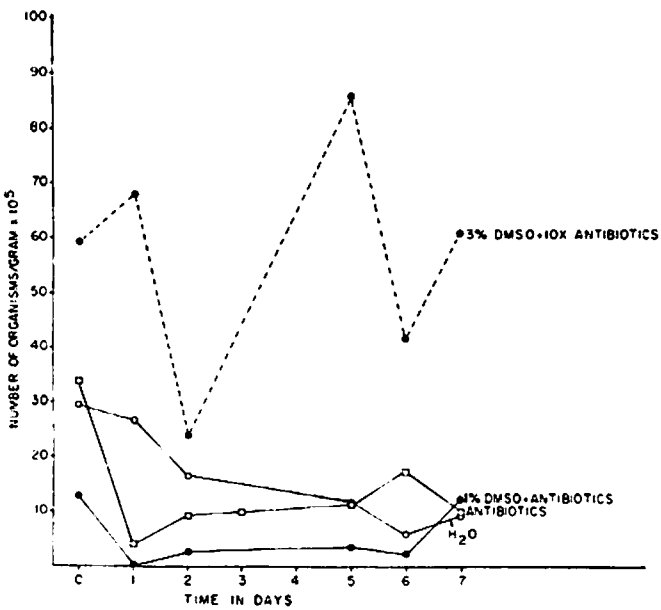

Figure 4. The change in total bacterial counts after surgical removal of the intestinal tract in diseased bullfrog tadpoles treated with DMSO and/or antibiotics.

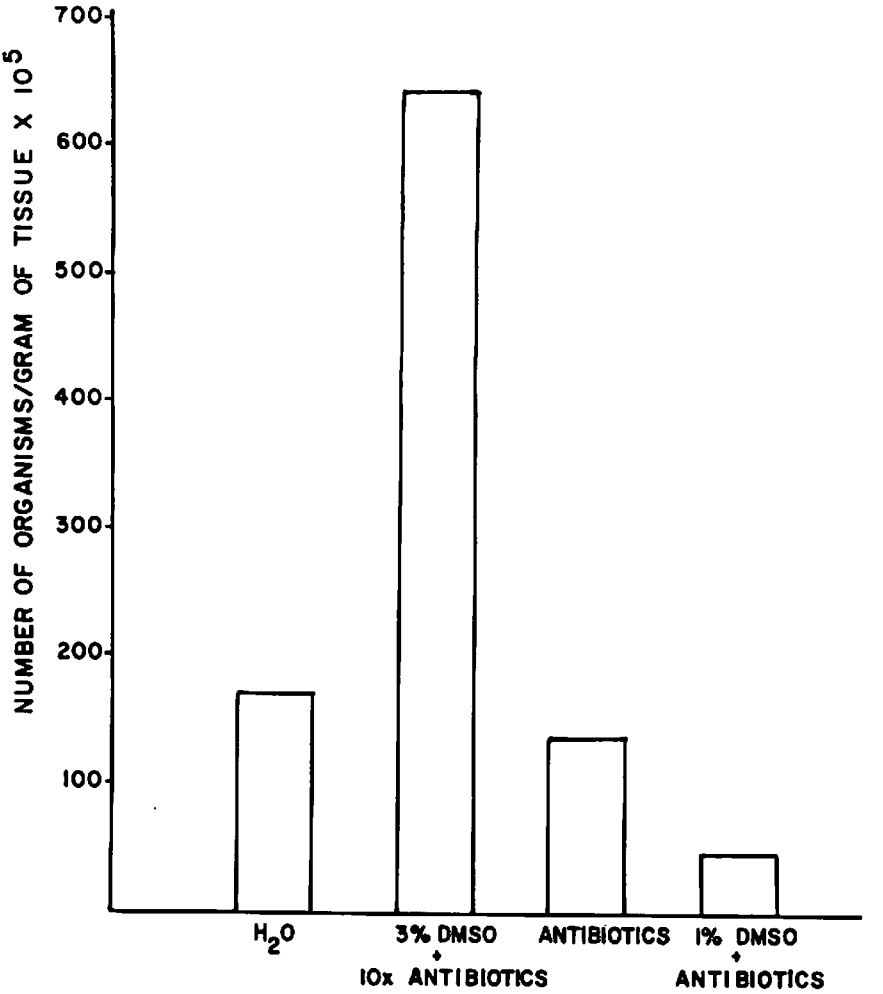

Figure 5. The total bacterial counts after surgical removal of the intestinal tract in diseased bullfrog tadpoles treated with DMSO and/or antibiotics averaged across the time period of treatment.

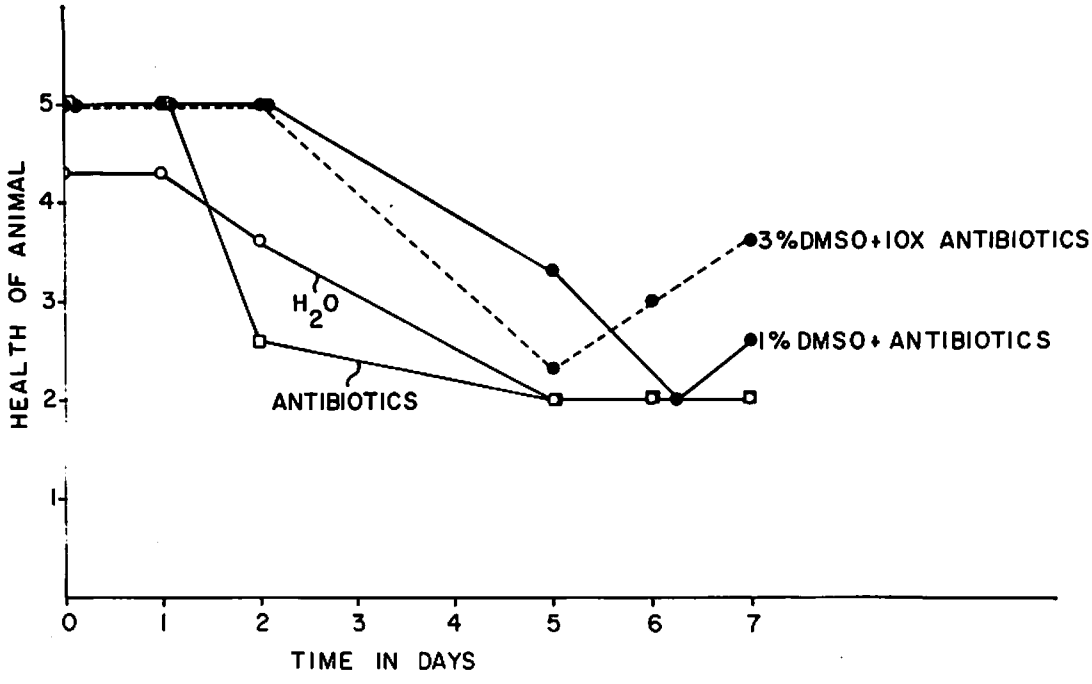

Figure 6. The changes in diseased bullfrog tadpole health during treatment with DMSO and/or antibiotics.

DEVELOPMENT OF AN ORGAN CULTURE SYSTEM FOR THE STUDY OF AN INVASIVE GRAM POSITIVE ORGANISM INFECTING BULLFROGS, Rana catesbeiana[1]

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INTRODUCTION

The one pathogen - one disease concept is a common occurrence, and in those instances where such specificity exists, the role of the pathogen can be probed by direct experimentation on the intact compatible host. However, as our knowledge of the disease process improves, it is becoming evident that complex interactions may stimulate or inhibit the physiological activities of bacteria which contribute to the induction of disease processes (Smith, 1972). In these instances, each member of the interacting system must be characterized before its role in the disease process can be explained.

Recent observations on frogs afflicted with red-leg disease indicate that the clinically important bacteria associated with this disease include 13 gram negative bacteria (Glorioso et al., 1974) and one gram positive bacterium (Amborski and Glorioso, 1973). Attempts to study the role of the gram positive isolate by using the intact frog proved to be difficult. In almost every case

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the interpretation of the results was limited by the presence of one or more of the identified gram negative organisms. This report presents our efforts to develop an organ culture system for the study of the gram positive isolate.

MATERIALS AND METHODS

Bacteria

The gram positive organism was isolated as previously described and maintained on Ordal Earp agar (Glorioso et al., 1974).

Organ Culture

Healthy frogs were sacrificed by pithing and fat bodies were removed aseptically. Small fragments of fat body, 5 mm to 10 mm in length were placed on grids in plastic organ culture dishes or were allowed to float on the surface of media in 60 cm^2 plastic tissue culture dishes (Bioquest, California). Sera, Eagles minimal essential medium (MEM) and Wolf and Quimby amphibian tissue culture medium (WQ) were obtained from Grand Island Biological.

RESULTS AND DISCUSSION

Glorioso et al. (unpublished results) demonstrated that the gram positive isolate invaded the fat body of frogs showing clinical symptoms of red-leg disease, and even in frogs near death none of the gram negative pathogens were ever observed to be invasive. Thus the fat body seemed to present a system in which the gram positive isolate could be studied without interference from the gram negative isolates. Figure 1 demonstrates a typical organ culture system. The fragment is floating free on the surface of the medium and the individual fat cells of the organ culture can be seen at higher magnification in Figure 2. The nature of the basal medium was apparently not critical as organ cultures in MEM, WQ or varying combinations of MEM and WQ showed similar responses. Furthermore, media supplements of bovine, fetal calf, horse and human sera at concentrations of 10 and 20 percent each gave approximately equal results. However, it must be emphasized that the lipid stored in each cell may have overcome any deficiencies in our media preparations and many of our observations could represent the effects of substantial endogenous activity. Long term cultures are presently being observed to provide information on this problem. It has been acknowledged by others (Monnickendam and Balls, 1973), however, that there is no evidence that amphibian cells or tissues normally require media fundamentally different from those used with avian or mammalian tissue culture systems.

Histological sections indicated that under the described

conditions, the structural integrity of the fat body could be maintained for periods of up to two weeks, after which time some central necrosis began to appear in some of the cultured fragments. Monnickendam and Balls (1973) have recently described amphibian organ culture systems which appeared normal and were still physiologically active up to 21 and 28 days in culture.

Based on these observations, fat body organ cultures were set up, and after 24 hours 10^6 of the gram positive cells were added to the tissue culture medium and allowed to remain in contact with the organ culture system for 24 hours. The medium was then removed and replaced with an equal volume of the same fresh medium, and incubation continued. Cultures subsequently showing contamination were discarded, and fat body fragments were prepared for histological studies at three day intervals. Figure 3 represents a section of an uninoculated organ culture system after 13 days in culture. The cell boundaries are intact and the nucleii appear normal. In contrast to these observations, Figure 4 represents a similar section prepared from an inoculated culture. Obvious cell destruction has occurred and foci of gram positive bacteria were observed. At higher magnifications as shown in Figures 5 and 6, these same effects are much more evident. Attempts to show increases in the numbers of bacteria by standard plate count techniques were unsuccessful. Nonspecific adsorption, clumping of bacteria, and small tissue size may have contributed to this problem.

During the course of these experiments, medium evaporation inadvertently increased the interaction of the fat body fragment and the surface of the plastic dish. Under these conditions the culture behaved as a classical explant culture, and cells as shown in Figure 7 were observed to migrate out from the fat body fragment. Ninety-eight percent of the attached cells contained lipid droplets as demonstrated by Sudan Black staining of similar cells grown on glass coverslips. These droplets were observed in three patterns:

1) A single large droplet

2) Numerous small droplets

3) Combinations of large and small droplets.

Attempts to infect these cells with the gram positive isolate were unsuccessful. Although gram positive bacilli could be demonstrated, we could not differentiate whether or not the bacteria were confined to the surface or had invaded the monolayer cells. Thus it may be possible that the three dimensional matrix of the organ culture may be required for infectivity.

LITERATURE CITED

Amborski, R. L., and J. C. Glorioso. 1973. The frog revisited. Science 181:495.

Glorioso, J. C., R. L. Amborski, G. F. Amborski, and D. D. Culley, 1974. Microbiological studies on septicemic bullfrogs, Rana catesbeiana. Am. J. Vet. Res. 35:1241-5.

Luna, L. G. 1968. Manual of histological staining methods of the armed forces institute of pathology. McGraw-Hill Book Co. New York. p. 222-3.

Merchant, D. J., R. H. Kahn, and W. H. Murphy. 1964. Handbook of cell and organ culture. Burgess Publishing Co. Minneapolis, Minnesota. p. 176-97.

Monnickendam, M. A. and M. Balls. 1973. Amphibian organ culture. Experientia 29:1-17.

Smith, H. 1972. The little-known determinants of microbial pathogenicity. In Microbial pathogenicity in man and animals (H. Smith and J. H. Pearce, eds.). 22nd Symposium of the Society for General Microbiology. Cambridge University Press, London. p. 1-24.

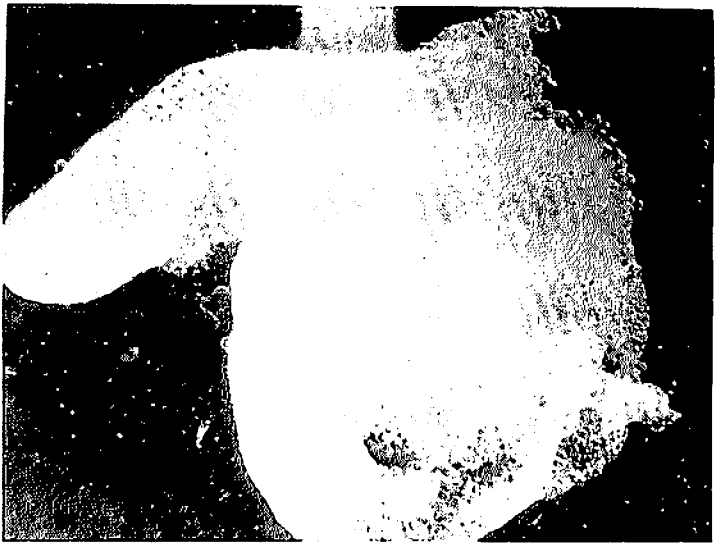

Figure 1. Organ culture of frog fat body. (10X)

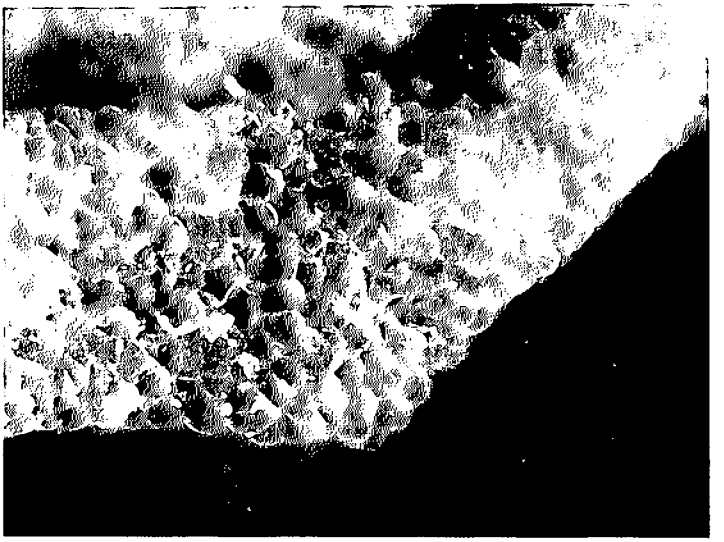

Figure 2. Organ culture of frog fat body. (50X)

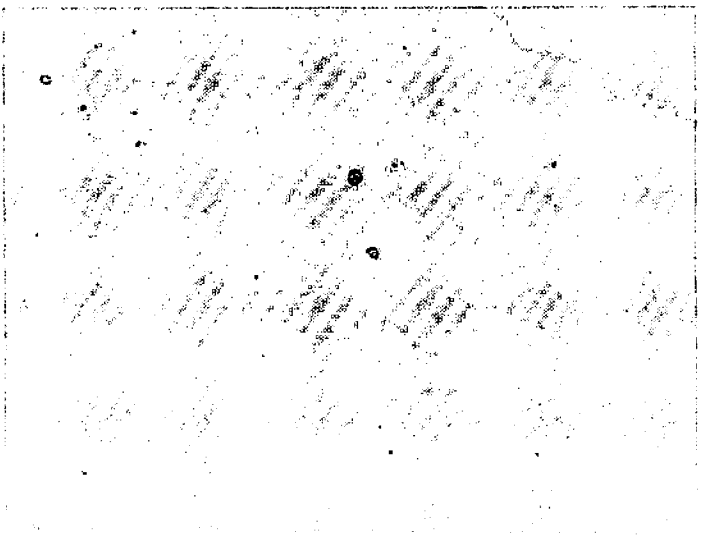

Figure 3. Histological section of frog body organ culture after 13 days incubation. Uninoculated. (200X)

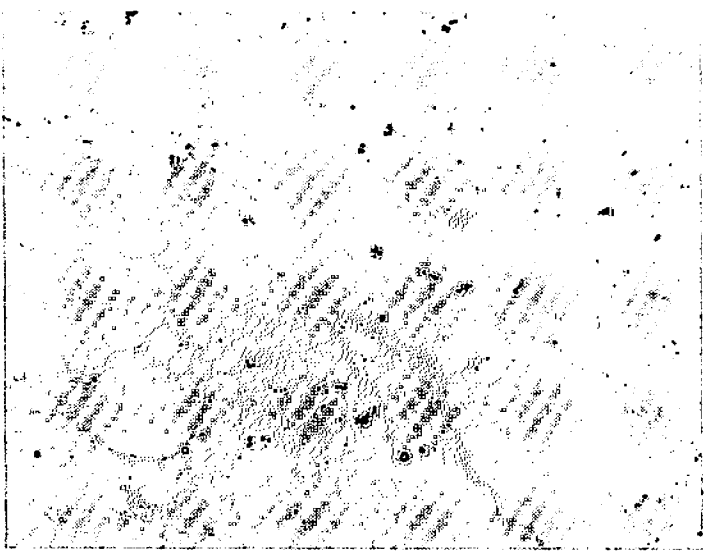

Figure 4. Histological section of frog body organ culture after 13 days incubation. Inoculated with gram positive isolate. (200X)

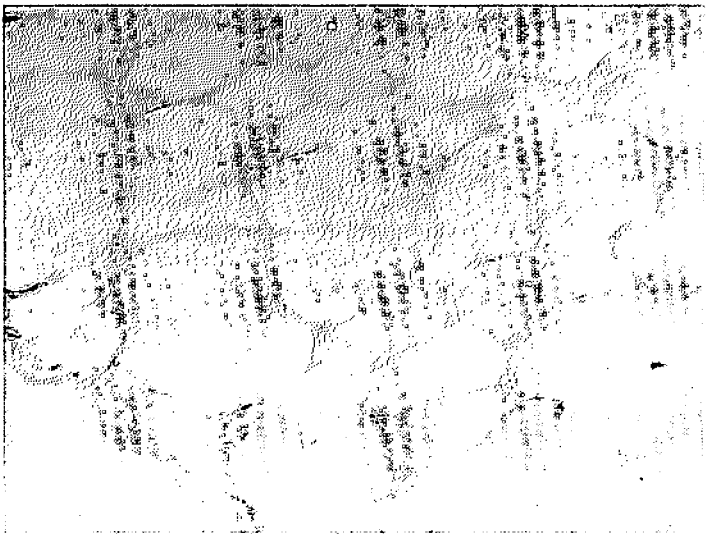

Figure 5. Histological section of frog body organ culture after 13 days incubation. Uninoculated. (800X)

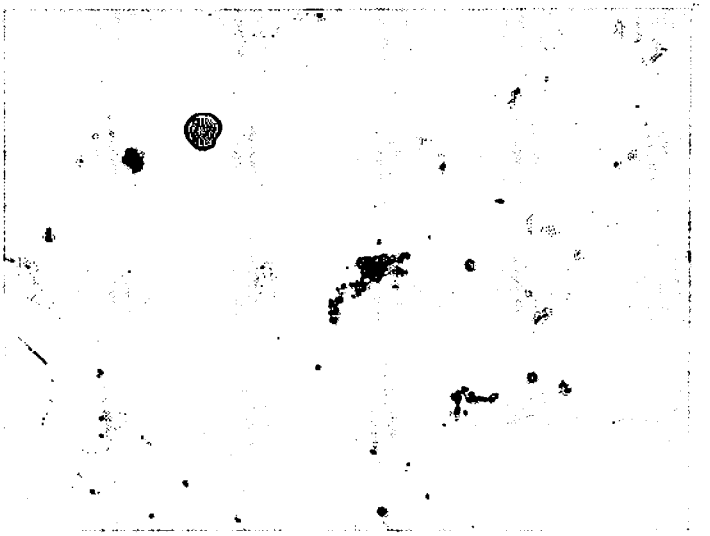

Figure 6. Histological section of frog body organ culture after 13 days incubation. Inoculated with the gram positive isolate. (800X)

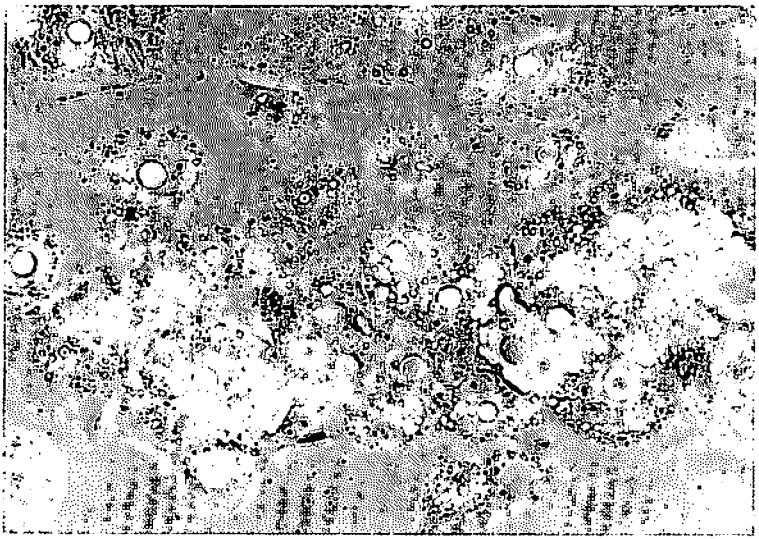

Figure 7. Phase contrast micrograph of outgrowth from explant culture of frog fat body. (700X)

DISEASES AND PARASITES OF *Xenopus*, THE CLAWED TOAD[1]

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INTRODUCTION

Various species of the genus *Xenopus*, South African clawed toads, have been popular experimental animals in zoology and embryology laboratories for years. They also have been used widely in pregnancy testing programs. There are some excellent references to their care and maintenance and their diseases and parasites, yet considering their wide usage, such literature is scanty.

For the last six years, some of the *Xenopus laevis* used in this laboratory for experimental zoology have been examined parasitologically (435 toads) and histopathologically (369 toads). The results of our examinations are summarized here and some of the more useful references to diseases and parasites of *Xenopus* are included.

MATERIALS AND METHODS

Originally, the toads were obtained from Jay Cook, importer, and apparently originated from the Capetown area of South Africa. About two years ago, we started receiving toads directly from the South African supplier. The toads are called *Xenopus laevis* (Daudin) but attempts to get a specific identification have so far been unsuccessful. Most of the toads used are purchased as adults, and the studies reported here are on the imported toads and not on those raised in our colony. Some successful breeding has been carried out over the years as a source for tadpoles and juveniles, however.

[1]Research supported by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation.

The toads are maintained in stainless steel tanks, approximately ten toads to a tank, with a dechlorinated constant flow water supply at 19 C. They are totally aquatic. They are fed weekly on chopped beef heart and liver. Tanks are cleaned the day after feeding. The average census of toads on hand is about 150.

Most of the toads are killed for experimental purposes by anesthesia and small portions of organs (liver, heart, ovary, etc.) or blood are removed before submitting the fresh remains for pathology. A relatively complete autopsy and examination for parasites is done. Samples of major tissues and organs as well as abnormal areas are selected for histopathology. Bouin's fixative is most commonly used. Slides are prepared by a technicon system and stained routinely with hematoxylin and eosin. Special stains are occasionally used for diagnostic problems, microorganisms, etc. Pathological and parasitological findings are recorded for each toad.

RESULTS

Table 1 lists the nonparasitic diseases found in the toads. Some of the more important or interesting will be discussed here. The generalized infections called "red-leg" were manifested by septicemia, scattered hemorrhages, and varying degrees of edema. Ideally, bacteriological studies should be done to try to determine the etiology. However, we are largely confined to pathological examination so we may be lumping several infectious diseases under the term "red-leg." Microbiological examinations were done on a few toads, and Aeromonas sp. (hydrophila ?), Proteus sp. (morgagni ?), and Edwardsiella tarda were cultured from heart blood. A number of epidemics of red-leg occurred in the colony, and many of the toads dying at those times were not examined pathologically; therefore, the tabulated incidence is low. Some cases of edema were not associated with apparent infection.

A few toads had great distention of the space between skin and body wall, so-called "gas infection" (Fig. 1). When the skin was incised under water, air or gas bubbles were released. There was no odor, and in the few attempts at culture, either no growth was obtained or a mixture of possible contaminants resulted. The source of the air or gas is unknown. Some affected toads were treated with an achromycin bath (125 mg/l) with good results.

Ten of the toads had tuberculosis which occurred as nodules in skin or some of the viscera (Figs. 2-5). Since the reaction is a very hyperplastic reticular granuloma, these cases were at first

thought to be some form of reticulum cell sarcoma. However, special stains revealed numerous mycobacteria in the lesions (Fig. 6).

Two toads developed rather widespread lymphosarcoma (Fig. 7). It involved muscle as well as viscera in one (Fig. 8). Another had a thigh tumor which resembled a fibrosarcoma with areas of necrosis, muscle invasion, and degeneration (Fig. 9). The only other possible tumor was a huge mass in a thigh (Fig. 10), which we called a chondroma. Another toad had a marked chondroblastic proliferation in the lower leg and foot which seems to be a reaction to injury.

The skin of the toad has several types of glands which undergo physiological variations. Degenerations of these glands was a common accompaniment of debilitating diseases, skin nematode infections (Fig. 11), and dermatitis. Some toads had localized skin ulcers with varying degrees of inflammation in edge and base.

In Table 1, most of the serosal disease consisted of atrophy of the abdominal fat bodies in sick or emaciated toads.

The urinary system was most frequently involved in disease processes. We used the term "nephritis" when kidneys had focal or generalized areas of edema, inflammatory cell infiltrate, and fibrosis or scarring. Nephritis occurred with or without cases of tubular degeneration, tubular casts, renal calcification, and glomerular hyalinization (Fig. 12). The relation of the tiny kidney nematodes to the various phases of renal disease was not clear. The urinary bladder in about one-fourth of the toads showed an epithelial degenerative change manifested by vacuolated "balloon cells" and some irregularity and hyperplasia (Fig. 13). In some of these cases Trichodina were present, but in many others they were not.

In addition to scattered visceral necroses in red-leg disease, we encountered a number of toads with single or a few small necroses of organs, especially the liver. In these cases the etiology was undetermined.

An uncommon but very interesting lesion was the formation of nodules in the intima of large vessels or on epicardial or serosal surfaces. There were six cases of these reticular granulomas. No organisms were demonstrable by fungal or mycobacterial stains. One lesion had a vague central body of indeterminate type (Fig. 14).

Table 2 lists the parasites encountered, their location and frequency, and the associated tissue damage. In the following paragraphs the names we use for the parasites are those that seem

most likely after a comparison of our specimens with descriptions in the literature.

Protozoa

The ciliate, Trichodina xenopodis Fantham, 1924, occurred in the urinary bladder of approximately 1/6 of the toads. It is a large, active protozoan and pulls a bit of mucosa into its attachment disc when at rest (Fig. 15). The relation of this parasite to bladder epithelial degeneration is not known.

A variety of ciliates were seen in toad feces (Fig. 16-18). We did not do enough fecal examinations to determine the incidence of these, and no attempts to identify them have yet been made.

Trematoda-Monogenea

Gyridotylus gallieni Vercammen-Grandjean, 1960, was first found in the preserving fluid of toads whose viscera were exposed. Further search revealed their presence in the nasal cavities of about 1/12 of the toads and several times they were found on the esophageal mucosa (Fig. 19). The strong posterior hooked haptor can attach to a bit of mucosa with slight physical damage (Fig. 20).

About one-fourth of the toads had Polystoma xenopi Price, 1943, in the urinary bladder (Fig. 21). The posterior haptor has six suckers and small hooks and causes slight physical damage at the site of attachment. The juvenile forms are found in the same organ.

Trematoda-Digenea

Larval forms of strigeid trematodes occured in one-half of the toads. They were free in body cavities (Fig. 22) including the pericardium and were encysted in a wide range of tissue locations (Fig. 23). Those examined were compatible with Diplostomulum xenopi Nigrelli and Maraventano, 1944. However, it is possible that there are several species. We did not encounter the form encysted in the lateral-line organs which has been noted in other studies. Several species of unidentified adult Digenea, including a Phyllodistomum (Fig. 24), were found in the intestine or gallbladder of about 1/20 of the toads.

Cestoda

Cephalochlamys naquamensis Cohn, 1906, a pseudophyllidean tapeworm, was the only adult species of cestode found and occurred in the intestine of about 1/20 of the toads (Fig. 25, 26). No tissue damage was associated with it. Slightly more frequently, we found unidentified larval cestodes in the intestine or in tissue cysts.

Nematoda

Camallanus kaapstaadi Southwell and Kirshner, 1937, was found in about one-half of the toads. This bright red nematode lies attached to the upper gastric mucosa causing slight damage where the mouth is applied (Figs. 27, 28).

A species of Capillaria was found in skin tunnels in about one-third of the toads (Fig. 29). This is by far the most pathogenic of the parasites encountered, causing extensive skin damage and irritation from the presence of worms and eggs in the tunnels. The surface of infected toads becomes rough and thickened, and they slowly become emaciated (Fig. 30). Undoubtedly, these skin lesions serve as a portal of entry for aquatic microorganisms. We speculate that the life cycle is a direct one, with ova in the skin sloughs being ingested by toads in the tank.

In marked contrast to findings in most other anurans, we found Rhabdias sp. in the lung only once.

In about 1/5 of the toads, an unidentified larval nematode was found in the kidneys upon histological examination (Fig. 31). It was usually in the glomerular space. Its association with the common renal lesions of these toads was not obvious. It is not known whether it reaches the glomerular by way of the circulation or the urinary tubules.

Several unidentified species of nematodes were found free in the intestine or in tissue cysts in about 1/20 of the toads. Some of these are larval forms, perhaps arising from the adults elsewhere in the body.

Acarina

The mite, Xenopacarus africanus Fain, 1969, was found in the nasal cavity of 11 toads. In some, the tiny, black, sluggish mite was seen alive in the nasal mucus when the cavity was opened.

In others, it was found in the nasal chambers histologically (Fig. 32). No lesions are ascribable to it.

DISCUSSION

Some information on diseases of Xenopus is scattered through the literature, with much summarized in reviews (Reichenbach-Klinke and Elkan, 1965; Walton, 1964 and 1966-1967). Balls (1965) and Elkan (1960) have had extensive experience with Xenopus colonies. Most descriptions of amphibian disease deal with neoplasms, with several general reviews (Balls, 1962; Balls and Ruben, 1968; Dawe, 1969; Lucke and Schlumberger, 1949; Reichenbach-Klinke and Elkan, 1965; Schlumberger and Lucke, 1948; Walton, 1964 and 1966-1967) and case reports (Balls, 1965; Elkan, 1960, 1963, and 1970; Ruben and Stevens, 1970), some of which deal with Xenopus. The problem of tuberculosis in Xenopus (and other amphibians) has been a serious one, both from the veiwpoint of animal colony management (Reichenbach-Klinke and Elkan, 1965; Schwabacher, 1959) and as a diagnostic problem in proliferative (pseudoneoplastic) disease (Dawe, 1969; Elkan, 1960; Ruben and Stevens, 1970). The chronic granulomata of lower vertebrates are very reticulohyperplastic in active stages and certainly resemble neoplasms in some cases.

With a few exceptions, the diseases we found in our toads are similar to those reported previously. Our common urinary tract diseases, nephritis, kidney degeneration, and urinary bladder epithelial degeneration, do not seem to have been reported.

Several general catalogs of parasites are good sources of information on Xenopus (Doss and Farr, 1969; Yamaguti, 1958-1963; Walton, 1964 and 1966-1967), and the text on lower vertebrate diseases (Reichenbach-Klinke and Elkan, 1965) is helpful. A new text on parasites of laboratory animals also has good listings for Xenopus (Flynn, 1973). The various individual parasite species occurring in Xenopus have been reported at intervals for years (Beverley-Burton, 1962 and 1963; Elkan, 1960; Hobson, 1965; Lom, 1958; Nigrelli and Maraventano, 1944; Pritchard, 1964; Southwell and and Kirshner, 1937), and the reviews above have included these reports. Of the parasites we encountered, the only ones which do not seem to be referenced in the literature are the skin capillarids, the kidney nematodes, the lung Rhabdias, and the intestinal trematode Phyllodistomum.

In general, the parasite spectrum in our toads seems to be very similar in the two groups, that is, those imported directly and those imported via an American distributor.

LITERATURE CITED

Balls, M. 1962. Spontaneous neoplasms in amphibia: a review and descriptions of six new cases. Cancer Research 22:1142-54.

Balls, M. 1965. The incidence of pathologic abnormalities, including spontaneous lymphosarcoma, in a laboratory stock of Xenopus (the South African clawed toad). Cancer Research 25: 3-6.

Balls, M., and L. N. Ruben. 1968. Lymphoid tumors in amphibia: a review. Progr. Exp. Tumor Research 10:238-60.

Beverley-Burton, M. 1962. Some monogenetic trematodes from amphibia in Southern Rhodesia including a new species, Polystoma mashoni, sp. n. from Bufo regularis (Reuss). J. Parasit. 48:752-7.

Beverley-Burton, M. 1963. Some digenetic trematodes from amphibians and reptiles in Southern Rhodesia including two new species and a new genus: Sarumitrema hystatorchis n. gen., n. sp. (Plagiorchiidae) and Halipegus rhodesiensis n. sp. (Halipegiade). Proc. Helminth. Soc. (Wash.) 30:49-59.

Dawe, C. J. 1969. Some comparative morphological aspects of renal neoplasms in Rana pipiens and of lymphosarcoma in amphibia. In Biology of Amphibian Tumors (M. Mizell, ed.). Springer-Verlag. New York. p. 429-40.

Doss, M. A., and M. M. Farr. 1969. Index-catalog of medical and veterinary zoology. Subjects: Trematoda and trematoda diseases. U. S. Government Printing Office. Washington, D. C.

Elkan, E. 1960. Some interesting pathological cases in amphibians. Proc. Zool. Soc. (London) 134:274-96.

Elkan, E. 1963. Three different types of tumors in Salientia. Cancer Research 23:1641-5.

Elkan, E. 1970. A spontaneous anaplastic intestinal metastasizing carcinoma in a South African clawed toad (Xenopus laevis Daudin). J. Pathol. 100:205-7.

Flynn, R. J. 1973. Parasites of laboratory animals. Iowa State Univ. Press. Ames, Iowa.

Hobson, B. M. 1965. Cold-blooded vertebrates, including Xenopus laevis. Fd. Cosmet. Toxicol. 3:209-15.

Lom, J. 1958. A contribution to the systematics and morphology of endoparasitic trichodinids from amphibians, with a proposal of uniform specific characteristics. J. Protozool. 5:251-63.

Lucke, B., and H. G. Schlumberger. 1949. Neoplasia in cold-blooded vertebrates. Physiol. Rev. 29:91-126.

Nigrelli, R. F., and L. W. Maraventano. 1944. Pericarditis in Xenopus laevis caused by Diplostomulum xenopi sp. nov., a larval strigeid. J. Parasitol. 30:184-90.

Pritchard, M. H. 1964. Notes on four helminths from the clawed toad, Xenopus laevis (Daudin), in South Africa. Proc. Helminth. Soc. (Wash.) 31:121-8.

Reichenbach-Klinke, H., and E. Elkan. 1965. The principal disease-of lower vertebrates. Academic Press. New York.

Ruben, L. N., and J. M. Stevens. 1970. A comparison between granulomatosis and lymphoreticular neoplasia in Diemictylus viridescens and Xenopus laevis. Cancer Research 30:2613-9.

Schlumberger, H. G., and B. Lucke. 1948. Tumors of fishes, amphibians, and reptiles. Cancer Research 8:657-753.

Schwabacher, H. 1959. A strain of Mycobacterium isolated from skin lesions of a cold-blooded animal, Xenopus laevis, and its relation to atypical acid-fast bacilli occurring in man. J. Hygiene 57:57-67.

Southwell, T., and A. Kirshner. 1937. On some parasitic worms found in Xenopus laevis, the South African clawed toad. Ann. Trop. Med. Parasit. 31:245-65.

Yamaguti, S. 1958-1963. Systema Helminthum. Vol. 1, The digenetic trematodes of vertebrates; vol. 2, The cestodes of vertebrates; vol. 3, The nematodes of vertebrates; vol. 4, Monogenea and Aspidocotylea; vol. 5, Acanthocephala. Interscience. New York.

Walton, A. C. 1964. The parasites of amphibia. Wildlife Dis. 39, 40.

Walton, A. C. 1966-1967. Supplemental catalog of the parasites of amphibia. Wildlife Dis. 48, 50.

Table 1. Nonparasitic diseases of Xenopus

Total toads examined pathologically = 369

Pathology	No. of Occurrences
General	
Injury	4
"Red leg"/Septicemia	18
"Gas infection"	3
Edema	13
TB	10
Skin	
Gland degeneration	100
Dermatitis	10
Ulcer	17
Fungus	2
GI Tract	
Infection	2
Prolapse rectum	3
GI Accessory	
Liver necrosis or degeneration	12
Gallbladder disease	4
Serosal disease	14
Urinary - Bladder	
Epithelial degeneration	85
Urinary - Kidney	
Nephritis	122
Degeneration, calcification, etc.	39
Gonad - Ovary	
Degenerating ova	7
Hemopoietic	
Hyperplasia	16
Cardiovascular	
Thrombosis	4
Intimal nodules	6
Tumor or Hyperplasia	5

Table 2. Parasites of *Xenopus*

Total toads examined parasitologically = 435

Type of Parasite	Location	No. of Occurrences	Associated Tissue Damage
Protozoa - *Trichodina*	bladder	73	mild
Ciliates	intestine	common	
Monogenea - *Gyrdicotylus*	nose, esophagus	38	slight
Polystoma	bladder	101	slight
Digenea - Strigeid metacercaria	various	218	some in tissue cysts
Adult species	intestine	27	
Adult species	gallbladder	2	
Cestoda - *Cephalochlamys*	intestine	21	
Larval	various	29	some in tissue cysts
Nematoda - *Camallanus*	stomach - esophagus	218	slight
Capillaria	skin	139	severe
Larval	kidney	52	slight
Other	cysts or intestine	21	some in tissue cysts
Rhabdias	lung	1	
Acarina - *Xenopacarus*	nose	11	

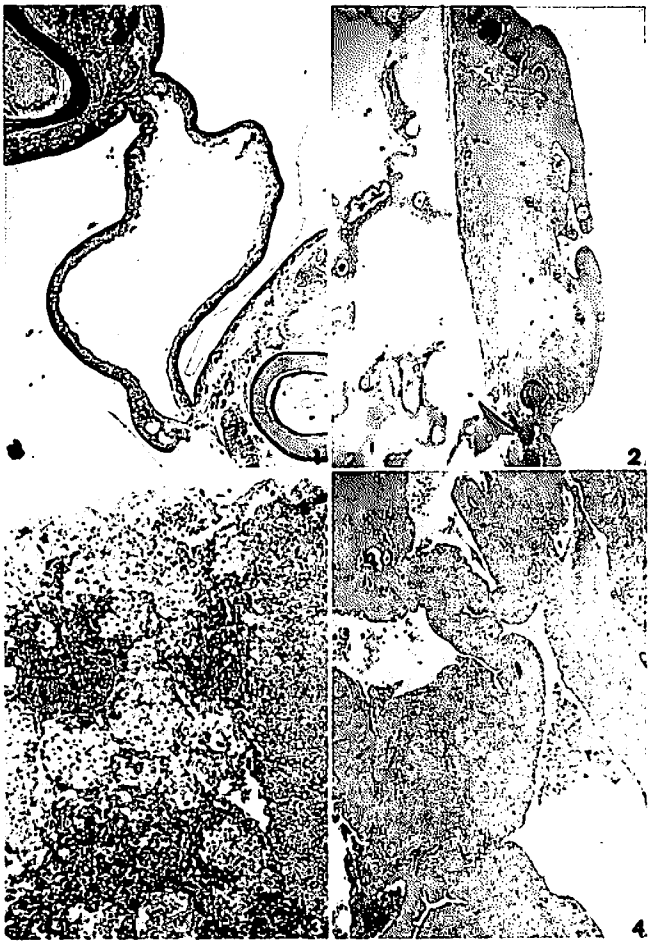

Figure 1. An interdigital web showing separation of the layers in "gas disease." (19X)

Figure 2. Tuberculosis of the skin. There is a raised ulcerated plaque with a core of granulomatous tissue. (19X)

Figure 3. Tuberculosis of the spleen. Scattered large and small granulomas are present. (76X)

Figure 4. Tuberculosis of the lung. The usually delicated alveolar walls are markedly thickened and granulomatous. (23X)

All histopathological figures, except Figure 6, are stained with H & E.

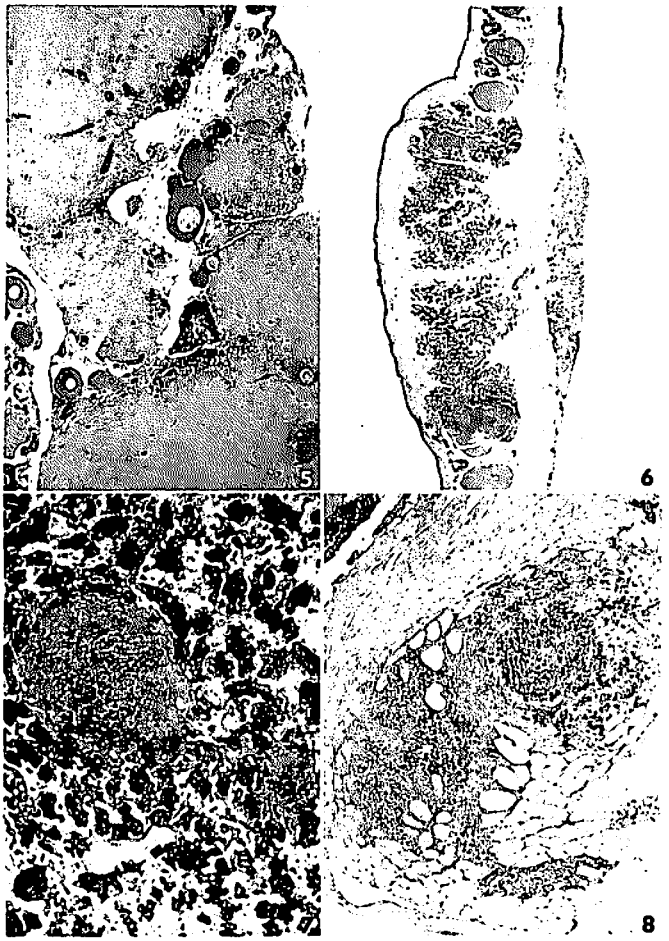

Figure 5. Tuberculosis of the ovary. Large masses of granulation tissue separate the groups of ova. (23X)

Figure 6. Tuberculosis of the skin: acid-fast stain showing masses of mycobacteria in the thick lesion. (19X)

Figure 7. Lymphosarcoma of the liver. One nodule and some smaller trabecular infiltrates are present. The black pigmented cells are normal. (30X)

Figure 8. Lymphosarcoma in leg muscle. (19X)

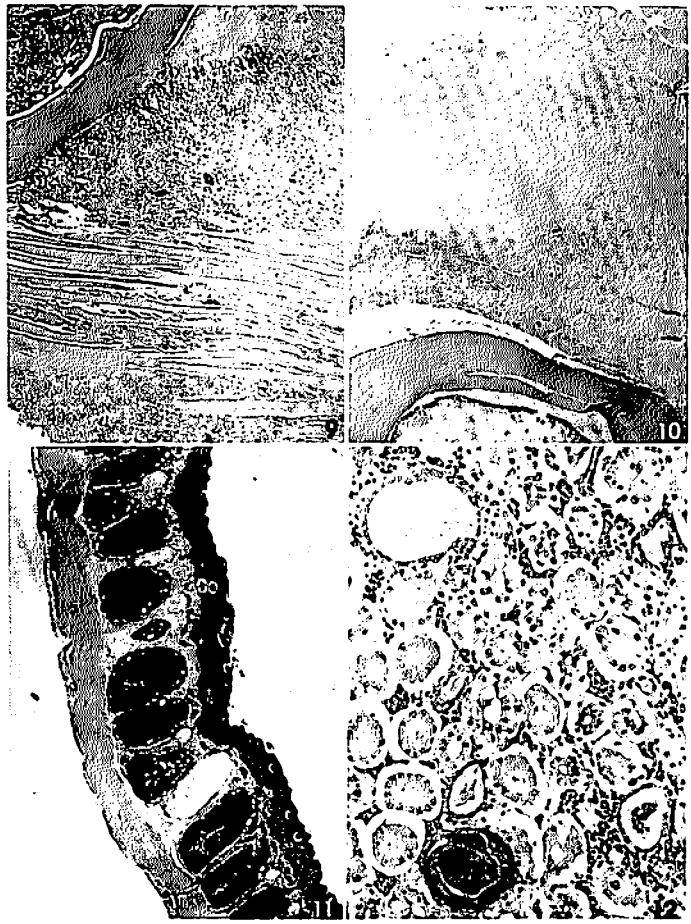

Figure 9. Fibrosarcoma (?) of the leg; infiltrating bone (upper left) and muscle (lower). (25X)

Figure 10. Chrondroma (?) of the leg. Femoral bone is at the lower edge. (25X)

Figure 11. Skin with capillarid nematodes in epidermal burrows. An unusual number of the dermal glands have granular degeneration. (40X)

Figure 12. Kidney with interstitial inflammatory infiltrate, one calcified area (dark), and one tubular crystalline deposit (light). (100X)

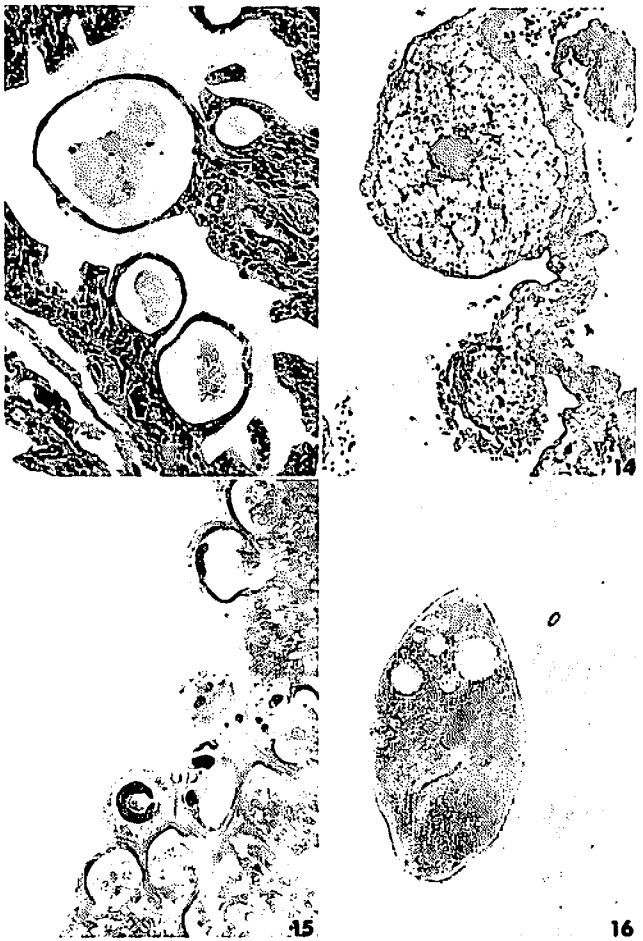

Figure 13. "Ballooning" degeneration of the epithelium of the urinary bladder. (80X)

Figure 14. Granulomatous nodules on the epicardial surface of the heart. (80X)

Figure 15. Trichodinid ciliates on the mucosa of the urinary bladder. Some show the C-shaped nucleus, the cup-shaped ventral surface, or the dentate ring. (256X)

Figure 16. An unidentified ciliate found in a fecal smear. (128X)

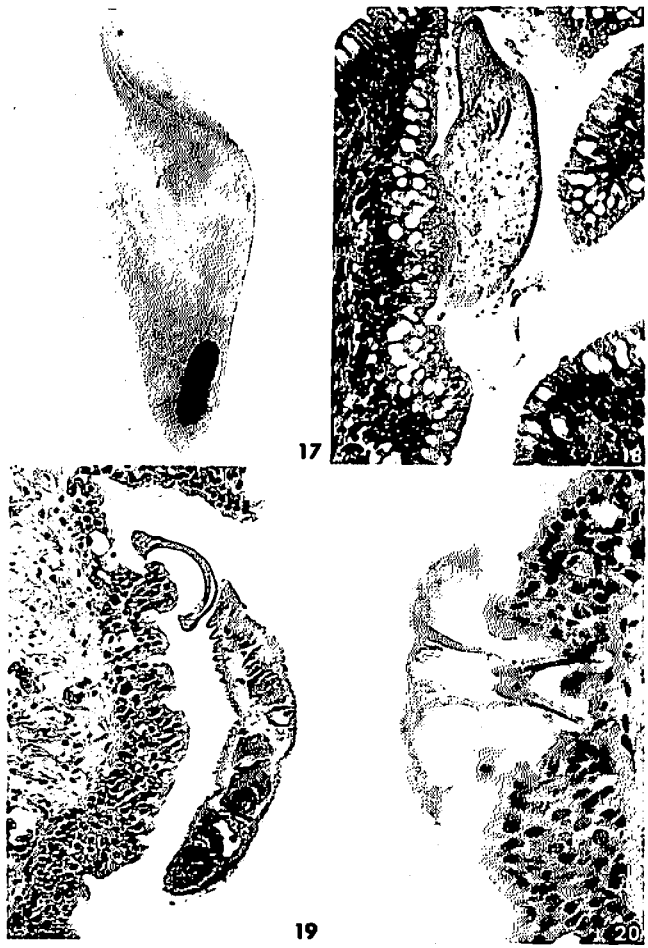

Figure 17. An unidentified ciliate found in a fecal smear. (128X)

Figure 18. The same species of ciliate as in Figure 17 adherent to the intestinal epithelium. (256X)

Figure 19. The monogenean, Gyridicotylus, attached to the esophageal mucosa by the posterior haptor. (200X)

Figure 20. The posterior hooks of Gyridicotylus embedded in the mucosa. (400X)

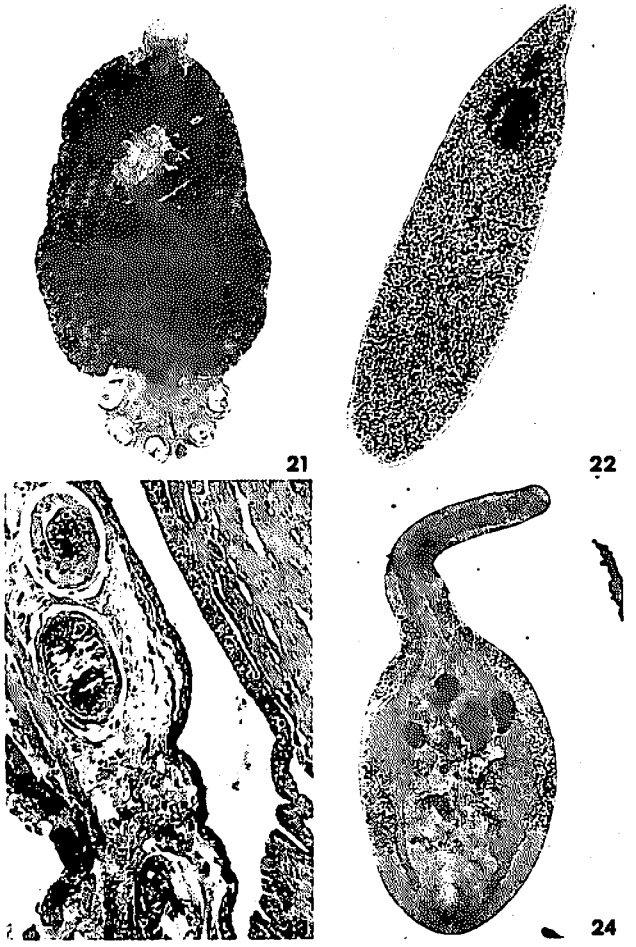

Figure 21. A whole mount of the monogenean, Polystoma, from the urinary bladder. (20X)

Figure 22. A whole mount of a metacercarial trematode from the body cavity. (80X)

Figure 23. Cysts of metacercarial trematodes in the tissue of the head. (100X)

Figure 24. A whole mount of a phyllodistomid trematode from the intestine. (8X)

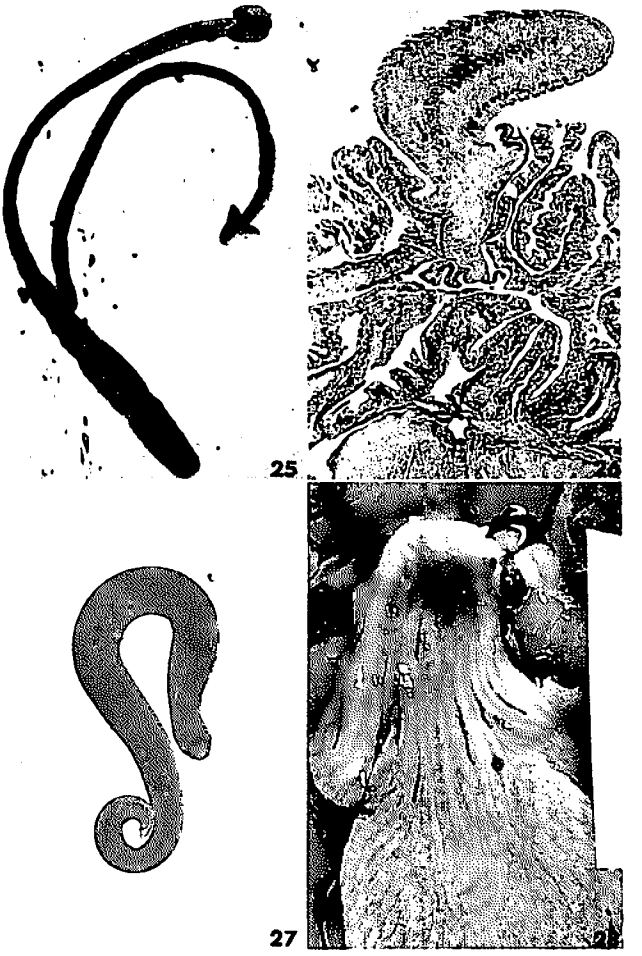

Figure 25. A whole mount of two tapeworms, Cephalochlamys, showing lateral and A-P views of the scolex. (8X)

Figure 26. Cephalochlamys attached to the intestinal mucosa. (80X)

Figure 27. A whole mount of a male nematode, Camallanus, from the gastroesophageal junction. (32X)

Figure 28. Camallanus nematodes in situ in the lower esophagus. (8X)

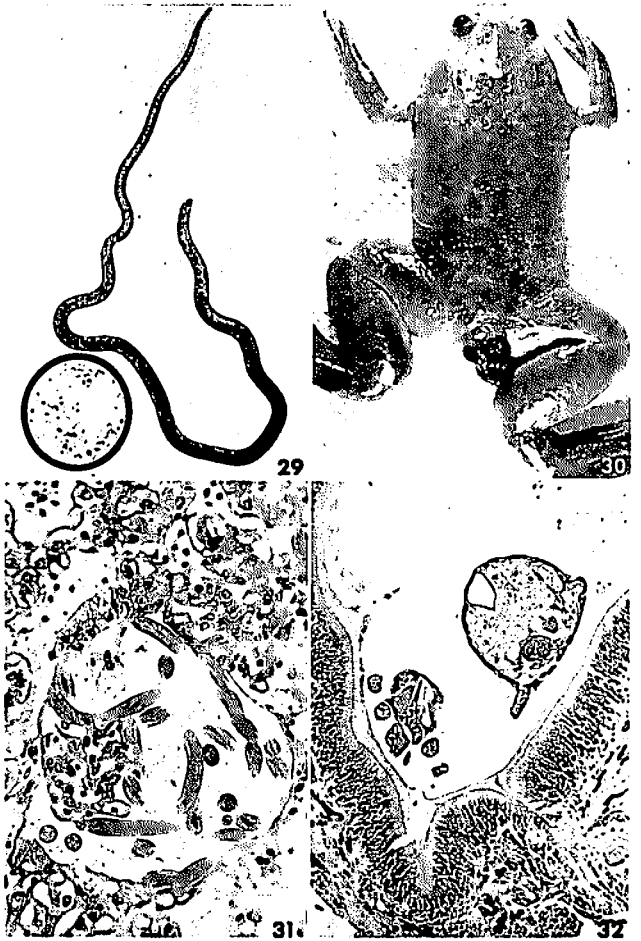

Figure 29. A whole mount of a female capillarid nematode from an epidermal burrow. (32X)

Figure 30. External view of a toad with skin nematodiasis showing the roughening and desquamation of the surface. This toad also has a rectal prolapse.(.5X)

Figure 31. Larval nematodes in the glomerular space of the kidney. (148X)

Figure 32. The nasal mite, Xenopacarus, in the nasal cavity. (40X)

PRELIMINARY OBSERVATIONS ON THE TREATMENT OF BABY TURTLES (Pseudeyms scripta elegans) FOR THE ELIMINATION OF Salmonella-Arizona

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INTRODUCTION

The red-eared slider (Pseudeyms scripta elegans) has been shown to be a carrier of Salmonella and Arizona which can cause human infections (Williams and Helsdon, 1965; Rosenstein et al., 1965; Kaufmann, 1966). Recent reports estimate that 300,000 cases of human salmonellosis which are associated with the presence of a pet turtle in the home occur yearly (Lamm et al., 1972). Because of the magnitude of the problem, both federal and state regulations have been implemented prohibiting the importation and interstate shipment of turtles infected with Salmonella or Arizona.

At the present, federal regulations require that lots of turtles must be certified free of Salmonella and Arizona before interstate shipment is allowed. The goals of this study are threefold:

1) To investigate the possibility of treating newly hatched "infected" turtles with antimicrobial agents and evaluate the feasibility of eliminating Salmonella-Arizona organisms systematically,

2) To examine water taken from three commercial breeding ponds for levels of Salmonella-Arizona throughout the breeding and egg laying season,

[1]This investigation was supported by the Louisiana Sea Grant Program, a part of the National Sea Grant Program maintained by the National Oceanic and Atmospheric Administration of the Department of Commerce.

3) To examine bacteriologically the visceral and sex organs of adult females for the presence of these organisms, thereby formulating the mechanism by which the baby turtle becomes infected and subsequently, becomes a carrier.

MATERIALS AND METHODS

Animals

Newly hatched turtles (Pseudeyms scripta elegans) were obtained from local turtle farmers (Pontchatoula, Jonesville, and Pierre Part, La.) as needed, were housed in plastic gallon containers in groups of 10, and were held at 65 F until needed.

Bacteriology

During the course of these studies various specimens were assayed for the presence of Salmonella and Arizona. Specimens studied included container water, whole baby turtle homogenate, egg homogenate, and visceral and sex organ homogenates after these tissues were removed from adult females. One ml of the above specimens was inoculated into 10 ml tetrathionate broth (Difco) containing 10 mg of brilliant green dye per liter (TBG), which was then referred to as the primary enrichment. Twenty-five ml of each of the homogenized samples were inoculated into 225 ml lactose broth (pre-enrichment), the pH was adjusted to 6.8, and they were incubated at 35 C for 24 hr and then subcultured into duplicate TBG. All TBG cultures were incubated at 35 C for 48 hr and were then streaked onto bismuth sulfite agar (Difco). After 24 hr incubation, two to three colonies were picked from each plate and inoculated into triple sugar iron agar, lysine iron agar, urea, malonate broth, KCN broth, and lysine decarboxylase broth. Salmonella and Arizona isolates were partially serotyped using commercially available polyvalent, group specific O antiserum, and phase 1 and phase 2 Arizona antiserum (Difco).

When the primary TBG enrichment was one week old, all TBG cultures which were negative for Salmonella-Arizona were subcultured to a second TBG, according to the method of Morris (1970) (secondary enrichment). These were then processed as discussed above.

Treatment of Newly Hatched Turtles with Antimicrobial Agents

Prior to treatment, each lot of turtles was assayed bacteriologically by the Food and Drug Administration certification and test

procedures (Federal Register, 1972) to determine if each experimental group was shedding Salmonella-Arizona into container water. Only turtles shown to be shedding these organisms were subjected to treatment with antimicrobial agents.

Experimental treatment and control groups consisted of 5 to 10 turtles. Turtles in each treatment group were exposed to neo-terramycin (NT) and terramycin (T) (Pfizer) which had been dissolved in container water to give 20, 100, 400, 800 or 1000 µg antibiotic per ml of water. The turtles were exposed for 1, 3, 6, 7, 9, 12, and 14 days to each of these antibiotics. The turtles were placed in 1000 ml beakers in 100 ml of freshly prepared antibiotic solutions which were changed daily for those treatments carried out for more than one day. Control groups were placed in daily changes of 100 ml sterile distilled water. Prior to the various testing protocols employed to detect Salmonella-Arizona, the container water was changed five to six times over a 48 hr period at the termination of treatment.

Homogenation of Specimens

After completion of treatment regimens, representative turtles were homogenized in a Sorval Omni-Mixer in an attempt to free Salmonella-Arizona from systemic positions. Five turtles (approximately 30 g) were placed in a sterile 200 ml stainless steel cup along with 30 ml of lactose broth (in later experiments TBG was used). The turtles were blended at 16,000 rpm for 2 min at 4 C. After blending, one ml of homogenate was inoculated into duplicate TBG and 25 ml into 225 ml lactose broth. Subsequent culturing was performed by the procedures described above.

Adult female turtles were captured in January, February, March, and April 1974, from three commercial ponds. Each of the 20 turtles examined was killed by decapitation. After surface sterilization, the plastron was removed and the following fluids and tissues were removed: urine, bile, whole gall bladder, kidney, spleen, stomach-small intestine-colon intact, ovary, immature eggs, and oviduct. A cloacal swab was also taken. The urine, bile, and cloacal swab were inoculated directly into TBG. The visceral and sex organs and eggs were blended separately in equal volumes of TBG, one ml of each was inoculated directly into TBG, and the remaining homogenate was inoculated into 225 ml lactose broth. Cultures were processed in the manner already described.

Most Probable Number (MPN) Determinations

Approximately 500 ml of water was collected at designated sites from three commercial breeding ponds (ponds A, B, and K) in the Pontchatoula, La. area in January, February, March, April, and May, 1974. The most probable number method used was previously described by Kaufmann et al. (1972) and was performed as follows: 10 ml, 1 ml, and 0.1 ml of each collection sample was added to five TBG for each dilution. The 10 ml aliquots were added to equal volumes of double strength TBG, and the 1 ml and 0.1 ml aliquots were added to 10 ml of single strength TBG. Further processing of these cultures was the same as for other specimens.

RESULTS AND DISCUSSION

Treatment Studies

In May 1973, we received 160 baby turtles which had been treated with neo-terramycin. There were 14 experimental groups, two of which were control groups, and each experimental group consisted of ten turtles. The turtles had been treated with either 20 or 200 µg NT per ml of container water for 1, 3, 6, 9, 12, or 14 days. The treatment experiments had been initiated by Dr. James Fowler (LSU extension entomologist) at a turtle farm in Pontchatoula, La. Upon receipt of the turtles in the laboratory each group was housed in a gallon container in 100 ml sterile tap water. Water samples were taken every week post treatment, at which time each experimental group was transferred to a new sterile container. The water samples were tested for the presence of Salmonella-Arizona. Each group was assayed weekly until all animals in each group had died.

Table 1 presents the results of this experiment. As can be seen both control groups, A and B, and groups treated for one day (20-1, 200-1) and for three days (20-3, 200-3) with either 20 or 200 µg/ml NT shed Salmonella serotypes C_1 and C_2 and Arizona into the container water continually throughout the period of testing. Water samples were assayed through 26 weeks at which time the turtles in all groups had expired. There was intermittent shedding of Salmonella-Arizona in groups treated for 6, 9, and 12 days throughout the 26 week period. Experimental group 200-14 which consists of turtles treated with 14 daily changes of 200 µg NT per ml of container water did not shed detectable numbers of Salmonella-Arizona throughout 12 weeks. Water samples were not collected from groups control A, control B, 20-1, 200-1, 20-3, and 200-3 for weeks 9 through 14 since prior to that time Salmonella-Arizona had been detected at almost every collection time.

These data (Table 1) point out a fact previously reported, namely that Salmonella-Arizona may be shed intermittently in the

turtle feces (Kaufmann et al., 1972). It appears that treatment of turtles with 200 µg NT per ml water for 9 and 12 days greatly depressed Salmonella-Arizona numbers in the turtle so that they either were not being shed or were being shed in numbers too low to be detected by assay of container water.

The next experiment was designed to answer three questions. First, does treatment of turtles with 200 µg NT per ml for 14 days eliminate these pathogens from a systemic position? Second, would homogenation of whole treated turtles permit detection of tissue-based Salmonella-Arizona which can not be recovered in the container water? Third, will pre-enrichment of 25 ml water or homogenate in 225 ml lactose broth allow, through pre-enrichment, the recovery of these organisms which might not occur by direct primary enrichment in TBG?

Table 2 presents the results of an experiment designed to answer these questions. Three groups of turtles shedding Salmonella (Jugs 55, 58, 59) were treated with 200 µg neo-terramycin per ml for 14 days, and three control groups (Jugs 56, 57, 60) were treated with water in a similar manner. After the 14 day treatment five turtles from each group (subgroup a) were homogenized and cultured for Salmonella-Arizona. Control groups 56a and 57a showed Salmonella D and B respectively in direct TBG cultures whereas pre-enrichment in lactose broth failed to show Salmonella or Arizona. Control groups 56b, 57b, and 60b were held in sterile water for 14 days post treatment, and water samples were taken at 48 hr and 14 days post treatment and assayed for Salmonella-Arizona. As can be seen in Table 2 Salmonella and Arizona were recovered from the water for each of the 3 control groups. Subgroups a from treated groups which were blended showed Salmonella D (subgroup 55a) and Arizona for subgroups 58a and 59a whereas water samples taken from subgroups 55b, 58b, and 59b were uniformly negative for these organisms at 48 hr and 14 days post treatment, substantiating the findings seen in experiment 1. The NT treatment eliminated shedding of Salmonella-Arizona into the container water but did not eradicate these organisms systemically.

These results show that 200 µg NT per ml of water reduces or eliminates shedding of Salmonella-Arizona into the container water but does not eliminate the systemic presence of these organisms. Homogenation, therefore, offers a good method by which to detect Salmonella-Arizona in the treated animal. Lactose pre-enrichment of homogenate is not superior to direct enrichment in TBG for the isolation of Salmonella-Arizona from turtle homogenates.

Since treatment with 200 µg NT per ml for 14 days suppressed shedding of these enteric pathogens the next experiment was designed to evaluate terramycin (T) at greater concentrations for treatment. Table 3 summarizes the results of an experiment in which

groups of turtles were treated with 100, 200, 400, or 800 µg T per ml of container water for either 7 or 14 days. Forty-eight hours after the treatment period was over all treated turtles in each group were homogenized and cultured by direct TBG and lactose pre-enrichment. The upper portion of the table presents the results for groups treated for seven days. Control groups (Jugs 9 and 10) were shedding serotypes F and C_2, respectively, prior to treatment, and the turtle homogenates revealed Salmonella serotype F by direct TBG enrichment. Homogenates cultured from groups 10 and 11 treated with 400 and 800 µg T per ml, respectively, were negative for Salmonella and Arizona, while those groups treated for 7 days with 100 (Jug 13) or 200 µg (Jug 12) were positive for Salmonella and Arizona in both direct TBG and lactose pre-enrichment cultures.

The lower portion of Table 3 shows the results of the 14 day treatment program. Again, as in the seven day experiment, Salmonella was isolated from control group turtle homogenates after 14 days treatment with water, both in direct TBG and lactose pre-enrichment cultures. Also Salmonella serotype B was recovered from TBG cultures of turtle homogenates prepared from those groups treated with 100 and 200 µg T per ml, but not from lactose pre-enrichment cultures. It is of interest to point out that the lactose pre-enrichment cultures were uniformly negative for Salmonella in these two groups while direct inoculation of homogenate in TBG were positive, whereas the lactose pre-enrichment cultures were positive for Salmonella and Arizona at the same terramycin concentrations in the seven day experiment. Also it did not always follow that the same serotype that had been isolated during pretreatment certification was recovered in treated or control group turtles after 7 or 14 days treatment. Of greater significance was the fact that groups 9 and 12 treated with 400 and 800 µg T per ml were positive for Salmonella upon homogenation. Turtles treated for seven days with the same concentrations of terramycin appeared to be negative; however, continued treatment with this agent through 14 days revealed that this was not the case. This may reflect the emergence of resistant strains or selection of a resistant serotype from an originally mixed serotype population.

Daily treatment with terramycin at doses of 800 µg per ml appeared to be initially hard on the turtle. Turtles were sluggish the first two to three days of treatment but appeared to become more active with time, and no deaths occurred through 14 days of treatment. Therefore, in the next experiment terramycin, neoterramycin, and tylosin (Ty) were incorporated into container water at 1000 µg per ml. Three groups of five turtles each (2, 3, 4) were treated for seven days with T, NT, and Ty and were homogenized 48 hr after treatment (Table 4). Groups 6, 7, and 8 were treated for 14 days with the same antimicrobial agents and homogenized 48 hr after treatment, and groups 10, 11, and 12 were treated for

14 days and homogenized 14 days post treatment. Parallel control groups (1, 5, and 9) were run concurrently. Table 4 presents the results of this experiment. The control groups (1, 5, and 9) were shedding Salmonella group D prior to treatment with water and all control groups yielded Salmonella or Arizona when homogenized at the termination time for each phase of the experiment. Again different serotypes emerged within the same experimental group, which may reflect mixed Salmonella infections and random sampling when colonies were picked. Tylosin (Ty) at this dosage (1000 µg/ml) was extremely toxic for the baby turtles, and most of the animals died within the first seven days. Tylosin did not supress Salmonella-Arizona numbers for they were isolated from the direct TBG and lactose pre-enrichment cultures. As was the case in the previous experiment, homogenates prepared from turtles treated with terramycin and neo-terramycin (1000 µg/ml) for seven days (Jugs 2 and 3) were negative for Salmonella and Arizona. Homogenates of turtles prepared 48 hr post 14 day treatment with terramycin were still negative, whereas Salmonella E serotypes were recovered from 48 hr turtle homogenates (direct TGB) from animals treated with 1000 µg per ml of neo-terramycin. Turtles treated for 14 days with NT and T and homogenized 14 days later showed detectable levels of Arizona in the neo-terramycin treated group (Jug 11), whereas turtles treated with terramycin alone were negative for Salmonella-Arizona upon primary TBG enrichment and lactose pre-enrichment. However, secondary TBG enrichment subcultures were positive for Arizona.

Terramycin apparently reduces Salmonella-Arizona numbers signigicantly--at least below detectability until 14 days post treatment, and then they were recovered only upon secondary TBG enrichment. Daily treatment with 1000 µg terramycin per ml was not lethal for any of the treated animals through 14 days; however, there was noticeable softening of the shell which indicates some toxicity. Whether treatment of infected baby turtles with higher concentrations of antimicrobial agents will eliminate Salmonella-Arizona is, at this time, questionable. Other antimicrobial agents are being tested and cannot be commented on at this time.

Bacteriological Examination of Adult Female Organs for Salmonella and Arizona

Adult female turtles were captured at three commercial breeding ponds, designated pond K, pond BA, and pond BB, located in the Ponchatoula, La. area. Seven animals were taken in January, February, and March from pond K. This pond is approximately 45m wide, 90m long, and 0.3-0.6m deep and contains approximately 12,000 adult turtles. The turtles were taken to the laboratory and housed in 20 gallon plastic garbage cans until used. Table 5 shows the results of the bacteriological assays performed on

various visceral and sex organs. All seven pond K turtles examined showed Salmonella and/or Arizona to be present in the tissues assayed. Six of the seven showed Salmonella or Arizona to be present in the urine and gastro-intestinal tract. The presence of these organisms in the urine may present a mechanism by which the egg becomes contaminated. The female, prior to laying a clutch of 6-16 eggs, takes in a large volume of pond water via the cloacae. She then crawls onto the pond bank in search of a suitable nest area. Upon selecting a quiet secluded spot, she urinates on the soil to soften the dirt, then with her hind legs excavates a shallow goose-necked hole, drops her eggs, fills the hole with dirt, urinates again on the covered nest, and packs the dirt firm with her legs and plastron. If the urine is contaminated with Salmonella-Arizona and if the pond water drawn into her bladder holds high numbers of Salmonella-Arizona (see Table 6), the urine-water soaked mud provides ample opportunity for contamination of the eggs.

Four of the seven immature egg batches removed from the oviducts of pond K turtles showed Salmonella and Arizona to be present when homogenized and cultured. This may however, only reflect surface contamination of the eggs since in two of these turtles (K-1-2, K-3-2) the oviduct was also positive for these organisms. So it is possible for the eggs to become contaminated (infected) with Salmonella-Arizona while passing through the oviduct. The kidney, liver, and gall bladder present possible sites for permanent infections (carrier) and thus a continued source for these organisms. Upon homogenation, Arizona is the predominant organism isolated from these tissues, which endorses the findings of Wells et al. (1974) in that it appears arizonae have a greater tendency toward systemic involvement than do salmonellae.

Pond BA is very similar to pond K in that it has the same dimensions and population density and is 18 years old. Five of the six turtles assayed exhibited primarily Arizona infections; however, the incidence of this organism was not as widespread throughout the tissues as in pond K turtles (Table 5).

Pond BB, by contrast, is only three years old, also measures 45 by 90 m, and ranges from 1.6 to 2.7 m deep. There are 6,000 adults in this pond. Only two of the six turtles tested showed salmonellae or arizonae. Turtles captured from the newer, deeper pond BB which contains a lower population density showed a lower infection rate when compared to the shallow, more densely populated ponds K and BA. The breeder stock in pond BB are also considerably younger, which may also be a factor. Although this data is based on limited sampling, it does point toward a tendency.

MPN Determinations for Ponds K, BA, and BB

Table 6 shows the Salmonella-Arizona MPN values obtained on water samples collected monthly from ponds K, BA, and BB. The water temperature ranged from 22 C in January to 18 C in February and then gradually rose to 29 C in May. Through May there was considerable algae present in pond K, little in pond BA, and none in pond BB. The most important fact to observe in this study is the much lower number of Salmonella-Arizona organisms per 100 ml water in the deeper pond BB. These numbers remained depressed through April and May, those months when the turtles become active. Courtship and egg laying also occur during these months and the turtle farmers commence feeding the turtles in April. All these activities stir the waters, which along with increased metabolic activity in the turtle and an increase in water temperature results in a month by month increase in the number of Salmonella-Arizona organisms in the shallow more densely populated ponds K and BA. These preliminary observations strongly suggest that deeper ponds and less crowding of the adult turtles can appreciably reduce the salmonellae-arizonae numbers in the pond water. The lower infection rate present in pond BB turtles may also be a factor in these data. Again these observations represent limited sampling but do show clear-cut differences.

CONCLUSION

Treatments of infected baby turtles with antimicrobial agents were done in an attempt to eradicate Salmonella and Arizona systemically from these animals so that a safe product can be presented to the consumer. Since the baby turtle, when hatched commercially, does not come into contact with the infected adult, or with Salmonella-contaminated pond water or bank soil, it was thought that the baby turtle offered a point at which to break the carrier (infection) cycle with little or no chance of reinfection. Although large concentrations of terramycin and neo-terramycin (1000 µg/ml) greatly depressed systemic levels of these pathogens, they were not eliminated. These findings unveil a potential hazard. Wells et al. (1974) reported that their studies showed that microbial assay of turtle container water for presence of Salmonella-Arizona proved to be as sensitive as their assay of turtle organ homogenates. This latter report dealt only with non-treated turtles and not treated turtles. Since many turtle farmers treat newly hatched turtles with substances such as copper sulfate and in some instances antimicrobial agents, it is possible for treated turtles to be certified "Salmonella-free" by State Health Laboratories when FDA regulations call for the testing of 72 hr container water. It has been shown in this study that turtles treated with terramycin or neo-terramycin at a concentration of 100 to 200 µg per ml for seven days may suppress or

temporarily eliminate shedding of Salmonella-Arizona into container water for several weeks, while the organisms are still present systemically in the treated turtle. Homogenation of the turtle proves to be a better method of assay than simple bacteriological assay of container water for presence of these organisms, especially if the "medical history" of the turtles is unknown.

The possibility of eliminating Salmonella-Arizona from the newly hatched turtle by treatment with antimicrobial agents is at this time questionable. Such treatment may result in the emergence of resistance transfer factors in Salmonella populations which persist in the treated animal, and these organisms could find their way into the human population. We have had some initial success with terramycin at higher concentrations, but it is too early to make any predictions.

The adult female population is grossly infected with Salmonella-Arizona, especially those turtles in the densely populated shallow ponds. The infected adult is a continuing source of salmonellae-arizonae contamination in the pond water and on the bank and the subsequent contamination of the eggs either in the oviduct or by contaminated urine. Kaufmann et al. (1972) attempted to treat a commercial breeding pond with copper sulfate at levels of two to five ppm from April to mid September. Although salmonellae-arizonae numbers in the treated pond water were greatly depressed eggs gathered from these ponds during the treatment period gave rise to hatchlings which were excreting Salmonella. Our studies suggest that deeper ponds (about 2.5 m deep) or less crowding might possibly achieve the same result. Although this does not in itself eliminate the salmonellae-arizonae organisms, the numbers are depressed, and fewer infected adults may result.

Salmonellosis in the turtle is an acute problem and attempting to clean and rid the pond of these organisms is futile. The point of attack must be directed at the link where reinfection is least likely.

LITERATURE CITED

Federal Register. 1972. 37. No. 224:25672-25673 section 72.26. Sat. Nov. 18.

Kaufmann, A. F. 1966. Pets and Salmonella infections. J. Amer. Vet. Med. Assoc. 149:1655-61.

Kaufmann, A. F., M. D. Fox, G. K. Morris, B. T. Wood, J. C. Feeley, and M. K. Frix. 1972. Turtle-associated salmonellosis. III The effects of environmental salmonellae in commercial turtle breeding ponds. Am. J. Epidemiol. 95:521-28.

Lamm, S. H., A. Taylor Jr., E. J. Gangarosa, H. W. Anderson, W. Young, M. H. Clark, and A. R. Bruce. 1972. Turtle-associated salmonellosis. I An estimation of the magnitude of the problem in the United States 1970-71. Am. J. Epidemiol. 95:511-17.

Morris, G. K. 1970. Evaluation of salmonella culture procedures. Proc. of the Amer. Assoc. of Avian Pathologists. Salmonella Workshop. Las Vegas, Nev.

Rosenstein, B. J., P. Russo, and M. C. Hinchliff. 1965. A family outbreak of salmonellosis traced to a pet turtle. New England J. Med. 272:960-1.

Wells, J. G., C. G. McConnel, and G. K. Morris. 1974. Evaluation of methods for isolating Salmonella and Arizona organisms from pet turtles. Applied Microbiol. 27:8-10.

Williams, L. P., and H. L. Helsdon. 1965. Pet turtles as a cause of human salmonellosis. J. Amer. Med. Assoc. 192:347-51.

Table 1. Treatment of baby turtles with 20 and 200 μg/ml of neo-terramycin for 1, 3, 6, 9, 12, and 14 days followed by weekly assay of container water for Salmonella-Arizona organisms

Expt'l Group (10 turtles)	Weeks Post Treatment (+ indicates positive assay)																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Control A	+	+	+	+	+	+	+	+							+	ND										
Control B	+	+	+	+	+	+	+	+							+											
20 - 1 day	+	+	+	+	+	+	+	+	Not Done (ND)						+	ND	+									
200 - 1 day	+	+	+	+	+	+	+	+							+	ND	+									
20 - 3 days	+				+	+	+	+							+	ND		+				+				
200 - 3 days				+	+	+	+	+							+	ND		+		+		+				
20 - 6 days			+									+		ND	+	+	+	+								
200 - 6 days									+	+	+	+	+	ND	+	+	+				+	+				
20 - 9 days			+	+	+	+	+	+	+																	
200 - 9 days				+										ND						+					+	+
20 - 12 days														ND												
200 - 12 days									+					ND	+	+	+									
20 - 14 days					+									ND	+	+										
200 - 14 days																										

Table 2. Treatment of infected baby turtles with 200 μg/ml of neo-terramycin for 14 days, followed by assay of turtle homogenates (subgroup a) and container water (subgroup b) for Salmonella and Arizona

Jug No.	Certification[a] Expt'l Group[b]		Group Serotype	Bacteriological Assay Post Treatment: Turtle Homogenate[c] TBG[d]	Turtle Homogenate[c] Lactose	Container Water 48 hr TBG	Container Water 48 hr Lactose	Container Water 14 days TBG	Container Water 14 days Lactose
56	Control	a	F	+ D	0				
		b	F			+ F	ND	+ F	ND
57	Control	a	B	+ B	0				
		b	F			+ B	ND	+ B	ND
60	Control	a	B	ND[e]	ND				
		b	B			+ Ariz.	ND	+ Ariz.	ND
55	200	a	C_2	+ D	0				
		b	C_2			0	0	0	0
58	200	a	B	+ Ariz.	+ Ariz.				
		b	B			0	0	0	0
59	200	a	C_1	+ Ariz.	+ Ariz.				
		b	C_1			0	0	0	0

[a] [b] Each group tested prior to treatment to determine Salmonella serotype being shed.
[c] Ten turtles per group; five per subgroup.
[d] Subgroup a was blended at 16,000 rpm for 2 min 48 hr post treatment.
[e] Tetrathionate broth with brilliant green added.
Not done.

Table 3. Treatment of infected baby turtles with 100, 200, 400, and 800 μg/ml Terramycin for 7 and 14 days, followed by bacteriological assay of turtle homogenates for Salmonella and Arizona

Jug No.	Certification[a] Expt'l Group[b]	Serotype	7 Day Treatment Group Homogenates: Direct TBG[c]	Lactose 1	Pre-enrichment 2
9	Control	F	+ F	+ F	+ F
14	Control	C_2	+ F	+ F	+ F
10	800	B, F	0	0	0
11	400	E_1	0	0	0
12	200	F	+ Ariz.	+ Ariz.	+ Ariz.
13	100	F	+ E_1	+ E_1	+ E_1
			14 Day Treatment Group Homogenates		
13	Control	E_1	+ Ariz.	+ F	+ B
10	Control	B, F	+ B	+ B, F	+ B
12	800	F	+ E_1	0	0
9	400	F	+ E_1	0	0
14	200	F	+ B	0	0
11	100	0	+ B	0	0

[a] Each group tested prior to treatment to determine Salmonella serotype sheded.
[b] Five turtles per group.
[c] Tetrathionate broth with brilliant green added.

Table 4. Treatment of baby green turtles with 1000 µg/ml of terramycin (T), neo-terramycin (NT) and tylosin (Ty) for 7 and 14 days, followed by assay of turtle homogenates for Salmonella and Arizona 48 hr after 7 and 14 day treatments and 14 days after 14 day treatment

Certification[a]			7 Day Treatment		14 Day Treatment			
			48 Hr Homogenate		48 Hr Homogenate		14 Day Homogenate	
Jug No.	Expt'l Group[b]	Serotype	TBG[c]	Lactose	TBG	Lactose	TBG	Lactose
1	Control	D	+ C_1	+ C_1				
2	T-1000	E_1, Ariz.	0	0				
3	NT-1000	D	0	0				
4	Ty-1000	D	+ C_1, Ariz.	+ C_1, Ariz.				
5	Control	D			+ H, I	+ D		
6	T-1000	D			0	0		
7	NT-1000	E_1, Ariz.			+ E_1	0		
8	Ty-1000	D, Ariz.			+ Ariz.	+ Ariz.		
9	Control	D					+ Ariz.	+ Ariz.
10	T-1000	Ariz.					+ D	0
11	NT-1000	D					+ Ariz.	+ Ariz.
12	Ty-1000	D					- all dead -	

[a] Each group tested prior to treatment to determine Salmonella serotype being shed.
[b] Five turtles per group.
[c] Tetrathionate broth with brilliant green added.

Table 5. Bacteriological examination of adult female turtle visceral and reproductive organs and fluids for the presence of Salmonella and Arizona

Turtle No.	Tissue Homogenates Tested								
	liver	gall bladder	G.I. tract	spleen	kidney	urine[a]	oviduct	imm. eggs	cloacal swab[b]
K-1-1	ND	0	0	ND	ND	+D,F	0	0	
K-1-2	ND	$+C_1$,D	+D,Ar	ND	ND	+D,C_2	$+C_2$,D	A Ar	+D
K-1-3	ND	$+C_1$	$+C_1$	ND	ND	$+C_1$,Ar	0	$+C_1$	0
K-2-1	+Ar	0	+Ar	0	+*	0	0	0	+Ar
K-3-1	0	0	+*	0	+Ar	+Ar	0	$+C_1$	$+C_1$
K-3-2	+Ar	+	+Ar	+Ar	+Ar	+Ar	+Ar	+Ar	0
K-3-3	0	0	+Ar	0	+Ar	+Ar	0	0	+Ar
BA-1-1	ND	0	0	0	0	0	+*	0	0
BA-1-2	0	0	+*	0	0	0	0	0	0
BA-3-1	0	0	+Ar	+H	0	0	0	0	+Ar
BA-3-2	+Ar	0	+Ar	0	0	+Ar	0	+Ar	+Ar
BA-4-1	0	0	0	0	0	0	0	0	+Ar
BA-4-2	0	0	0	0	0	0	0	0	0
BB-1-1	ND	0	0	ND	0	0	0	0	0
BB-1-2	0	0	0	0	0	0	0	0	0
BB-1-3	0	0	+Ar	ND	+Ar	0	0	0	0
BB-3-1	0	+	0	0	0	0	+	0	0
BB-4-1	0	0	0	0	0	0	0	0	0
BB-4-2	0	0	0	0	0	0	0	0	0

[a] 25 ml urine was inoculated directly into 225 ml lactose broth.
[b] The cloacal swabs were inoculated directly into 10 ml tetrathionate broth.

Table 6. Most probable number (MPN) determinations performed on water collected from three commercial breeding ponds (K, BA, and BB) for Salmonella-Arizona levels for January through May, 1974

	MPN per 100ml Pond Water					
Pond	Jan 12	Feb 15	Mar 15	April 3	April 23	May 16
Pond K_1[a]	33	140	13[d]	ND[e]	920	240
Pond BA_1[b]	46	79	170	33	220	33
A_2	49	95	210	49	240	240
Pond BB_2[c]	2	33	24	2	1.8	4.5
B_5	0	33	17	4.5	23	7.8
B_{RO}	2	70	11		47	7.8

[a] Water collected at one foot depths.
[b] Water collected at one foot (A_1) and two foot depths (A_2).
[c] Water collected at two feet (B_2), five feet (B_5) and run-off (B_{RO}).
[d] Low value probably reflects the result of $CuSO_4$ treatment.
[e] Not done.

DETECTION OF INTERACTIONS BETWEEN NATURAL PATHOGENS AND POLLUTANTS IN AQUATIC ANIMALS[1]

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Hopefully, it is now generally accepted that the "germ theory" of disease applies to aquatic vertebrates and invertebrates as well as to terrestrial forms. That this has not always been the case is best illustrated by the fact that in the past when mass mortalities of aquatic animals occurred, the general ecologist often overlooked the possibility that infectious pathogens might have been the etiologic agent. More often than not, every other possible avenue of cause and effect was explored before a search for infectious disease agents was launched.

Presently, in this age of environmental consciousness when mass mortalities of aquatic animals occur, one of the first causes to be searched for is pollution. This is justified, of course, based on documented evidences that water quality and aquatic ecosystem stability have been lowered significantly in the last three decades by increasing industrialization, population growth, and water misuse.

Thus, those of us who must consider health of aquatic species have, on the one hand, infectious diseases and, on the other, pollution of waters, coming together as one complex of environmental factors that affect health in aquatic ecosystems. Impinging upon

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the complex of aquatic disease and pollution are, of course, other environmental factors such as temperature, pH, salinity, oxygen content, available nutrients, and agents of mechanical trauma. These too must be considered in evaluating the health of an aquatic species.

Only recently has the possible interaction of infectious diseases and pollutants, as environmental complexes, been seriously consided as threats to aquatic life. Snieszko (1972) has recently reviewed fish diseases that are heavily dependent upon environmental interactions. His general summation could be applied to any group of interacting environmental complexes, particularly infectious diseases and pollution:

> Modern epidemiology is based on the premise that epidemic outbreaks are caused by an imbalance between the host, the pathogens or other disease agents and the environment. Aquatic cold-blooded animals are much more affected by the environment than are the terrestrial homeotherms. Therefore, outbreaks of various diseases of fish are strongly affected by ecologic factors.

The purpose of this paper is to give two examples of the detection of possible interactions between natural pathogens and chemical pollutants in selected Gulf of Mexico, estuarine animals. These will include results of both experimental laboratory work and field observations in the vicinity of Pensacola, Florida.

Nimmo et al. (1971) have used pink shrimp (Penaeus duroarum) (Fig. 1) as test animals in toxicity studies for several years at the Gulf Breeze EPA laboratory. Recently, Couch (1974a) described a new virus in pink shrimp. It was observed during light and electron microscope studies of the hepatopancreas of toxicant exposed, control and feral shrimp (Figs. 2,3). The virus was found in hepatopancreatic epithelial cell nuclei (Fig. 4), is rod-shaped, and is either free in the nucleus or occluded in patent infections in crystalline inclusion bodies that range from 0.5 µm to 20 µm in size (Figs. 5,6).

The shrimp virus, named Baculovirus penaei by Couch (1974b), is considered to be a nuclear polyhedrosis virus because it shares many characteristics with the NPV or Baculoviruses (subgroup A) of insects (Wildy, 1971). It is the first Baculovirus reported in a host other than insects. The virus occurs in feral pink shrimp and is enzootic in populations of shrimp from Apalachee Bay, Florida and Santa Rosa Sound, Florida. It has been found most prevalent during the fall and winter months, occurring in from 0-30 percent of shrimp in given samples.

Pink shrimp from natural populations with enzootic levels of the virus have been used as subjects in toxicity tests of the PCB,

Aroclor^{R} 1254, and the organochlorine insecticide, Mirex. Shrimp exposed to 3 µg Aroclor/1 in flowing seawater for from 30 to 50 days accumulated up to 500 mg Aroclor/kg in their hepatopancreatic tissues. After approximately 30 days exposure, 50 percent of these shrimp died (Nimmo et al., 1971). From these series of tests, surviving, exposed shrimp were examined histologically and 60 percent were found to be lightly to heavily infected with the nuclear polyhedrosis virus. Control shrimp were free of patent virus infections. In another exposure test, conducted by Tagatz (personal communication), 38 percent of shrimp exposed to 0.01 to 0.23 µg commercial Mirex/1 of flowing sea water for 30 days demonstrated patent virus infections. Only 6.6 percent of control shrimp for this experiment showed patent infection. Mortality of shrimp in the Mirex exposure experiment was 81 percent, whereas the controls had only a 9 percent mortality. Thus, the prevalence of virus infections in the chemically exposed and control shrimp corresponded to the level of mortalities in those respective groups.

Another interesting finding has been that samples of pink shrimp kept under abnormally crowded conditions for 30-40 days in aquaria have shown 40-50 percent virus prevalence as compared to initial prevalence of 0-10 percent at onset of captivity (day one of holding period). This strongly suggests that the stress and proximity of crowding for a period of time enhances or facilitates the virus infection. Transmission of the virus from individual to individual via cannibalism in densely crowded aquaria or culture containers should be expected. In nature, even on fertile fishing grounds, shrimp are rarely as densely found as under aquacultural or aquarium conditions. Further, in nature dilution of infective stages of the virus by several factors (water volume, predation of infected shrimp by non-shrimp predators, etc.) would be greater than in closed less voluminous, artificial systems.

Therefore, as an example of a model system, we have the apparent enhancement of the prevalence of a Baculovirus by certain potential environmental factors, including chemical pollutant stress (PCB's and Mirex) and crowding. A similar model system of interactions between a toxicant chemical and a virus for higher vertebrates was presented by Friend and Trainer (1970), whose research demonstrated enhancement of duck hepatitis virus by polychlorinated biphenyls.

Numerous casual and careful observations of natural, aquatic ecosystems have led us to believe that where one finds chronic low level pollution (both natural nutrients and synthetic chemicals), one also observes increasingly frequent epizootics of certain diseases and a gradual increase in prevalence of certain pathogens in stressed hosts. This is not true, however, for every pathogen since some parasites may be adversely affected by the pollution as much as, or more than, their hosts. Each disease agent-pollutant

complex must be considered separately as well as part of more complicated, larger systems.

Over the last 10 years, we have observed in Escambia Bay, Florida, high prevalence of fin rot syndrome associated with mortalities in croakers (Micropogon undulatus) and spot (Leiostomus xanthurus) during periods of warm weather and oxygen depletion. Escambia Bay has been contaminated with the PCB, Aroclor 1254, for several years (Duke et al., 1970) and is a rapidly eutrophying system. Although we have not attempted to isolate pathogens from healthy or moribund fish, the patterns of disease and their seasonal occurrences strongly suggest a bacterial etiology. In this regard, Schwartz (1974) found that the bacteria Aeromonas and Pseudomonas, representative well-known fish pathogens, were found in higher prevalences in fish from Clear Lake, Iowa, in warmer seasons than in cooler seasons. In the laboratory during warming months of the year (April through June), we have been able to induce fin rot syndrome, identical to that in fish from Escambia Bay, in up to 90 percent of spot exposed to 3-5 µg/l of Aroclor 1254 (Couch, 1974c). This fin rot was associated with high mortality (80 percent), but again, no attempts were made to isolate a bacterial pathogen.

Thus, there is strong empirical and circumstantial evidence which suggests that interactions between natural pathogens and pollutants probably occur (Snieszko, 1974). Further research must determine the extent of the threat of such interactions to aquatic life and ecosystems. The Gulf of Mexico and its northern and eastern estuaries provide numerous natural sites for study of such interactions.

LITERATURE CITED

Couch, J. 1974a. Free and occluded virus, similar to Baculovirus, in hepatopancreas of pink shrimp. Nature 247:229-31.

Couch, J. 1974b. An enzootic nuclear polyhedrosis virus of pink shrimp. I. Ultrastructure, prevalence, and enhancement. J. Invert. Path. (in press).

Couch, J. 1974c. Histopathologic effects of pesticides and related chemicals on the livers of fishes. In The Pathology of Fishes. Univ. of Wisconsin Press (in press).

Duke, T., J. I. Lowe, and A. J. Wilson, Jr. 1970. A polychlorinated biphenyl (Aroclor^{R} 1254) in the water, sediment, and biota of Escambia Bay. Florida Bull. Environ. Contam. and Toxic. 5:171-80.

Friend, M., and D. O. Trainer. 1970. Polychlorinated biphenyl: interaction with duck hepatitis virus. Science 170:1314-16.

Nimmo, D. R., R. R. Blackman, A. J. Wilson, and J. Forester. 1971. Toxicity and distribution of Aroclor 1254 in the pink shrimp Penaeus duorarum. Marine Biology 11:191-97.

Schwartz, J. J. 1974. Prevalence of pathogenic pseudomonad bacteria isolated from fish in a warmwater lake. Trans. Amer. Fish. Soc. Vol. No. 114-16.

Snieszko, S. F. 1972. Progress in fish pathology in this century. Symp. Zool. Soc., London 30:1-15.

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

Wildy, P. 1971. Classification and nomenclature of viruses. First report of the international committee on nomenclature of viruses. Monographs in Virology 5:1-81.

Figure 1. Dorsal view of pink shrimp (Penaeus duorarum) with dorsal cuticle removed to show hepatopancreas in situ (arrow).

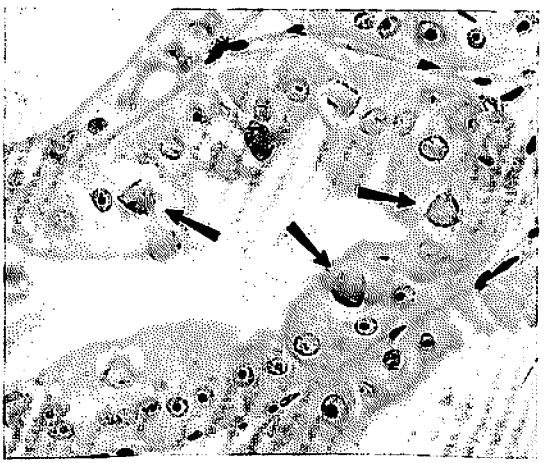

Figure 2. Cross section of shrimp hepatopancreatic tubule or acinus showing epithelial cell nuclei containing triangular baculovirus inclusion bodies (usually one per nucleus if cell is infected; arrows); normal nuclei are small with prominent nucleoli (820X). Feulgen, picromethyl blue stain.

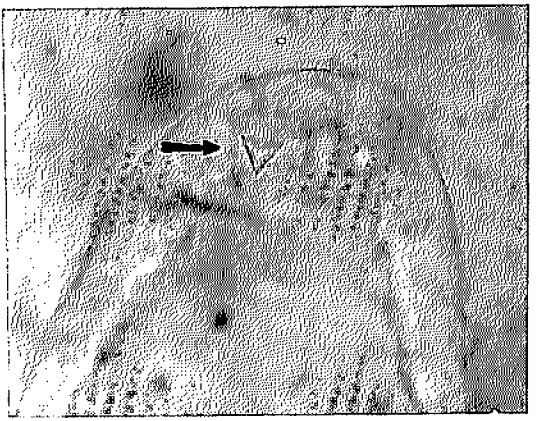

Figure 3. Fresh squash preparation of end of hepatopancreatic acinus; note single patently infected cell with large inclusion body that has the form of a tetrahedron (820X). No stain.

Figure 4. Section of hepatopancreatic epithelium showing cell with viral inclusion body in nucleus (arrow). (1230X)

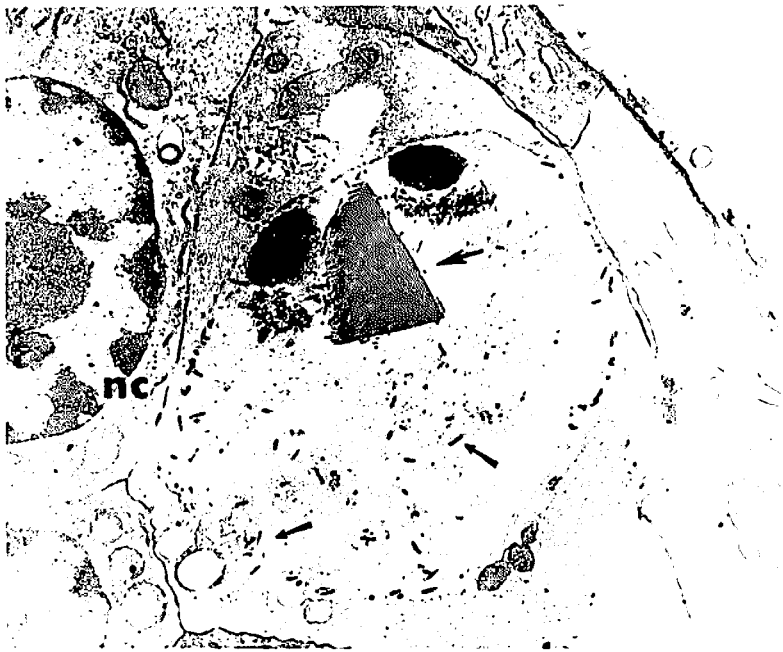

Figure 5. Electronmicrograph of virus infected hepatopancreatic epithelial cell from PCB-exposed shrimp; note triangular section of virion-containing inclusion body in infected cell; note virions free in nucleoplasm (arrows). Compare normal cell (nc) with infected cell (7800X).

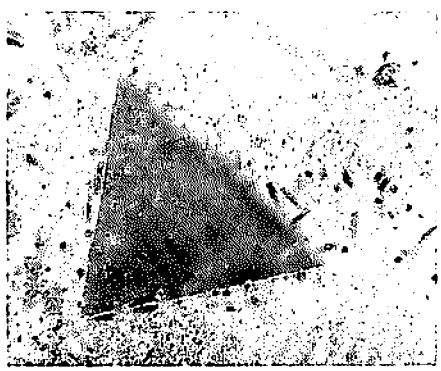

Figure 6. Higher magnification of inclusion body in shrimp cell. Note virions in longitudinal and in cross section embedded within matrix of characteristic triangular inclusion body (15,400X).

A SURVEY OF DISEASE RESEARCH REQUIREMENTS IN MARICULTURE[1]

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The presentations at this Symposium have given an excellent insight into the technical aspects of many entities that infect and kill some of the aquatic animals being evaluated as potential mariculture species. Communication and transfer of this technology is difficult because mariculture research is being conducted by diverse groups in numerous state, federal, and university laboratories as well as by some commercial mariculture firms on a pilot production scale. The following discussion is an analysis of a written survey conducted early in 1974 which attempted to define some of the disease problems in specific areas of the developing mariculture industry. The approach was to determine what species were being raised by whom, what were the problems, the periodicity of the problems in the life cycles of the animals, and the level of economic loss to the industry. I have attempted to blend the information received from this survey with the results of a national survey of mariculture conducted by the Mardella Corp. (1973) and with the technical information presented at this Symposium to propose a pragmatic mariculture management and disease consulting program. The major question is whether or not such a service is really needed at this time.

I recently joined the University of Miami from a private mariculture venture which raised freshwater and marine fishes, shrimp,

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[1] Contribution from the Rosenstiel School of Marine and Atmospheric Science, University of Miami.

clams, and oysters. I have experienced many if not all of the problems of attempting intensive culture of adult and juvenile marine animals with a minimum of technical information. All biologists in private mariculture ventures share the same problems.

From my practical experience with mariculture and disease problems in aquatic animals, it is my opinion that the major component of mortality in commercial mariculture is simply the lack of knowledge of how to keep marine animals alive in captivity. Many mass mortalities that I have experienced or investigated resulted from poorly designed culture systems and lack of knowledge of the biological requirements of the animals selected for culture. MORTALITY IS a real problem in mariculture. However, disease is being cited as the cause of death without giving any consideration to system design, technical expertise of personnel, carrying capacity of warm salt-water, water quality, food, source of brood stock, or the viability of marine animals in confinement.

Some of my opinions and the results of the survey I conducted conflict with the 1973 Mardella report, which was rather specific in its analysis of the relationship of disease to other problems in commercial mariculture. The report stated "... disease was identified as the greatest single obstacle to commercial aquaculture. Clinical assistance was urgently requested by operators... The limited U.S. competence in saltwater animal pathology was noted..." The impact of these findings upon potential commercial investors in mariculture has also become a great deterrent to industrial development in mariculture. Disease is one of the first subjects raised by investors. The general public does not understand host-parasite relationships and the role of preventative medicine in minimizing losses of animals.

The conclusions contained in the Mardella report were developed from a well-planned system of 12 regional workshops throughout the United States (Table 1). Approximately 25 percent of the participants represented commercial aquaculture operations. A large number of the participants consisted of administrators from state and federal agencies associated with the utilization of natural aquatic resources. Few, if any, of these persons have reared large numbers of animals in intensive culture systems. There were differences in the numbers of participants by geographic regions as well as differences in problems within each area. For instance, the West Coast participants were strongly oriented toward culture of salmonids, which do have serious disease problems from *Vibrio*, *Mycobacteria*, and viruses. Massive losses of shellfish on the West Coast were also of great concern. However, several well staffed federal and state laboratories exist to handle these problems.

In the survey Dr. E. S. Iversen and I conducted in the fall of 1973, we attempted to restrict our analysis to persons involved in aquaculture of species other than trout, salmon, and catfish, since these disciplines are served by numerous laboratories with a large number of well trained personnel. Reports from Auburn University and from Fish Farming Experiment Station (Bureau of Sport Fisheries and Wildlife) at Stuttgart, Arkansas, have delineated

problems in the catfish industry. Disease problems in the salmonid culture industry have been adequately surveyed and reported in Hester (1973).

In addition to the selection by species, survey questionnaires were sent to persons known or suspected to be actively involved in the growing of large quantities of marine animals or plants. Table 2 shows the geographic distribution of the survey questionnaires sent out and returned. Many people who were not participants in the Mardella workshop were contacted since our personal files supplemented the Mardella list. Additionally we extended the opportunity to all participants in the survey to make a confidential report. Many firms and persons involved in mariculture activities consider their work to be proprietary and will not divulge details for public distribution. About 15 percent of the replies were proprietary.

The Gulf States and the West Coast are more equally balanced in our survey, and we tried to contact more commercial firms in the Caribbean where mariculture is a rapidly developing industry. My colleagues in the Gulf States returned a high percentage of the questionnaires, but I still received replies from only about 75 percent of the persons contacted. The questionnaire was sent to 42 individuals considered to be in commercial mariculture in the United States, but we received only 18 replies. The discrepancy between these numbers and the 66 commercial participants of the Mardella workshop (Table 1) is explained by the facts that:

1) Only one representative from each firm was sent a questionnaire;

2) There was a dramatic reduction in some of the less viable mariculture firms during the 1973 change in the U.S. financial and economic picture; and

3) Commercial ventures were reluctant to provide written proof of their disease problems.

The participants at this workshop must realize that basic research must eventually have a consumer who can show an economic benefit from the scientific work. One contribution could be in personnel to execute disease control measures in large populations of aquatic animals. With such a low number of mariculture firms defined by this survey and by the Mardella report, it is difficult for me to endorse the Mardella finding that there is an immediate need in mariculture for more persons trained in diseases of saltwater animals. In fact, the number of participants in this workshop outnumbers the commercial firms who responded to a questionnaire which indicated that someone would provide diagnostic services to them if they would describe their problems.

Originally I planned to analyze the results of this questionnaire by computer data retrieval but due to the low number of replies and the simplicity of the answers, I only developed generalizations from the data. Virtually all of the disease entities described on the survey replies have been adequately discussed by speakers at this symposium. A host-parasite list would be redundant. Table 3 lists the genera of animals and plants grown in the various geographic areas of the United States.

On the West Coast (Table 3) salmonids and oysters are being reared in significant commercial quantities. Other species are in various stages of laboratory or pilot production study. The salmonid diseases that were reported were typical for intensive culture situations with Vibrio and virus diseases causing the greatest concern. In the shellfish and other invertebrates, problems in rearing larval stages were predominant in the reasons for mortality.

In the Northeast and Middle Atlantic a completely different group of animals is being considered for mariculture. Shellfish disease laboratories and disease specialists are supported by several state universities and at least two federal agencies. Very well equipped laboratories at Oxford, Md., and at Milford, Conn., have diagnostic programs to assist the shellfish industry. Culture of the northern lobster, Homarus, has several specific disease problems that occur on the West Coast as well as in the New England states. Gaffykemia, shell disease, and fungal infections were involved. The greatest losses in all species being cultured in the Northeast were associated with larval culture.

Moving away from the continental United States, to the Hawaiian and Caribbean Islands we discover areas where industrial mariculture is developing rapidly. Many mariculture firms in Hawaii are in pilot production stages and most of the reported problems were in the rearing of larval stages. It is highly probable that these problems are related to culture systems, nutrition, and utilization of wild brood stock.

The green sea turtle, Chelonia, is a highly suited candidate for mariculture in the islands. Commercial ventures are experiencing severe disease problems from a Herpes virus, coccidiosis, devective hatchlings, nutritional problems, and intensive culture system design. Penaeid shrimp are being raised by several ventures in the Caribbean since many of the problems of mass larval culture and nutrition have been solved in the past two years. Some of the disease problems that were reported at this meeting for penaeid shrimp, also affect Macrobrachium, being reared in both areas.

Regarding the animals being cultured in the Gulf States, the survey could add nothing to the series of fine reports of specific disease entities presented at this workshop. Most of the commercial

activity in the Gulf area is directed towards the Louisiana Crayfish. The penaeid shrimp and Macrobrachium industry is just evolving. Studies of other animals are largely experimental, and many of the problems are related to the design of culture systems and the lack of specific information on the culture requirements during the early life stages. Many of the fin-fish culture experiments were conducted in estuarine environments with poor water quality. Problems in shellfish have all been described in the scientific literature. Dermocystidium and environmental conditions such as red tide and anaerobic water seem to be the predominant cause of massive losses in oysters.

The final page of the questionnaire contained a question concerning disease inspection and quarantine of aquaculture products. The question was: "If current federal legislation concerning inspection and quarantine of fish and marine animals is passed, indicate the numerical order (1, 2, 3, etc.) of acceptability of the below agencies to conduct the inspection and to initiate quarantine action on your aquaculture product". Apparently most persons were unaware that there are numerous bills in Congress to enact legislation concerning quarantine inspection of mariculture products. Most replies were marked "not applicable", but from 30 replies, the agencies were ranked as to their desirability to conduct this service (Table 4). A score of 1 indicates the first choice and rank rating is in numberical mean of all the questionnaire ratings.

Most survey participants chose one of the existing agencies which has furnished some type of disease diagnostic service to them in the past. Due to the marine orientation of the group answering the questionnaire, National Marine Fisheries Service of NOAA was selected over the Bureau of Sport Fisheries and Wildlife of the Department of Interior. However, if members of the commercial trout and catfish industry were questioned, the ranking of these two agencies might have been different. State Universities did not fare well, and private consultant firms so often mentioned by government agencies as the preferred agency to do this job were the least desirable.

Perhaps this survey (Table 4) and the geographic distribution of mariculture operations (Table 3) will have some impact on the planning for quarantine inspection services. I anticipate that future mariculture disease research requirements will be closely integrated with a disease inspection agency. Quite logically mariculture disease research should be conducted by this inspection agency or by other groups under contract to that agency.

In analysis of questionnaire responses and in my own experience, high mortality during culture of early life stages of marine animals was the point of greatest concern. This was especially true

in marine fishes and some species of Crustacea. Often these losses resulted from not providing the proper environment and food of an adequate size, type, and quantity.

In many commercial operations and laboratory studies there was a strong desire expressed to do things differently, perhaps for patent reasons or to produce more animals per unit area or per volume of water than the existing literature recommends. Both of these procedures have caused dramatic losses to researchers and commercial mariculture ventures.

To illustrate some of the problems involved in rearing early life stages of marine animals, Table 5 shows the success of 33 production scale culture "runs" using penaeid shrimp at the University of Miami's Turkey Point shrimp culture facility from 1969 to 1974. I excluded the first year of operation since that was a "learning experience" and was plagued with high losses of animals. Nineteen shrimp culture attempts had severe losses. From our records and careful observation we determined that biological failure and human error were the major reasons for losses. Disease was implicated in only two instances. Biological problems included lack of egg viability, failure of larvae to develop normally, loss of food supply, and predation of early stages by a contaminant or predator. Equipment failure combined with biological problems and human error indicates that penaeid larval culture requires well trained scientific and maintenance personnel. Culture of penaeid shrimp is a "green thumb" or "fishy thumb" art rather than a scientific manufacturing process. The most important conclusion from these data is that a high percentage of time losses do occur but they are not related to disease. However, I recognize that disease entities will increase in number and become a problem under conditions of poor husbandry. Productivity in penaeid larval culture facilities can be easily increased by system analysis, proper training, and on-site disease and water quality surveillance.

Following penaeid larval shrimp culture into the "pond grow-out" phase, the frequency distribution of survival in 49 pond culture studies conducted at the Turkey Point facilities is shown in Table 6. Even though the ponds are located in the extreme southern portion of the United States, they experience "winter kill" especially when we attempt to grow Penaeus duorarum or P. occidentalis between 15 November and 15 March. As shown in Table 6, 8 out of 23 winter pond culture attempts had very high mortality and 11 out of 23 would have been economic disasters. In the summer months ponds at Turkey Point have severe water quality problems caused by excessive plankton blooms, biological oxygen demand from shrimp food, and lack of water exchange (Krantz and Iversen, 1974).

During harvest of the experimental grow-out ponds, the University technicians and staff conducted extensive examination of shrimp for disease problems. We have only rarely found "cotton shrimp" or "shrimp black spot" disease in our ponds. With the severe environmental problems caused by improper pond design and poor pond management concepts, survival was acceptable in only 18 out of 49 pond experiments at Turkey Point.

In summary, this survey and the research experience at Turkey Point experimental mariculture facility indicate that many of the losses of animals in mariculture occur in their early life stages. Disease can cause significant mortality in specific animals and under specific conditions, but there is little evidence that disease is the greatest single deterrent to mariculture.

There are a limited number of commercial mariculture operations in the Gulf coast region, too few to support a commercial consulting firm. Their problems seem to be related to the culture system design and the lack of detailed information on how to satisfy the biological requirements of mariculture candidates. It appears that many mortality problems in mariculture can be solved by integrated research programs of a system analyst, an engineer, a disease specialist, and a well-trained culturist. From the fine presentations at this workshop, the major disease entities involved in mariculture losses are being studied by competent investigators.

Any mariculture disease condition reported in the survey of the Gulf States could be solved by the utilization of the skills and knowledge that have been shown by the group assembled at this workshop. The mechanism by which this highly developed technology can be transferred to the problem area is not clear, but it is easy for me to create a hypothetical management consultant agency with disease specialists to assist commercial mariculture in the solution of its disease and mortality problems. This group may be a government agency or a well funded research group at a university. I am presently attempting to establish such a program at the University of Miami. This program will be multidisciplinary and will integrate disease research specialists in other university laboratories. Services would be available to non-commercial mariculture programs at no charge.

Even though some of the information collected during this survey is proprietary, workshop participants may obtain from me the names of firms who have specific disease problems or specific non-proprietary information within the mariculture industry.

ACKNOWLEDGMENT

I wish to thank Dr. E. S. Iversen, University of Miami, for assisting in the development of this questionnaire. I am deeply indebted to my wife, Lucretia, for her scientific expertise and diligence in conducting the preliminary data analysis for me. I want to thank all of those who participated in this survey for assisting in the advancement of knowledge in the area of marine pathology.

LITERATURE CITED

Hester, E. F. 1973. Fish health: A nationwide survey of problems and needs. Prog. Fish Cult. 35(1):11-18.

Krantz, G. E., and E. S. Iversen. 1974. Husbandry helps harvest healthy shrimp. Gulf and Carribean Fish. Inst.

Mardella Corp. 1973. Summary report of NOAA regional aquaculture workshops.

Table 1. Geographic distribution of participants in Mardella aquaculture workshops (from January 1973 Report).

Area	Total Number	Commercial Orientation
Northeast	34	8
Middle Atlantic	27	5
Gulf Coast	78	17
West Coast	94	29
Caribbean	2	2
Hawaii	23	5
TOTAL	258	66 (25.6%)

Table 2. Geographic distribution of disease survey questionnaire and replies

Area	Number Sent	Number Received	Number of Commercial Firms
Northeast	33	19	1
Middle Atlantic	23	15	3
Gulf States	64	44	4
West Coast	62	24	6
Caribbean	5	4	3
Hawaii	13	8	1
TOTAL	200	114	18

Table 3. Geographic distribution of genera in mariculture (*commercial venture)

West Coast	Middle Atlantic
*Oncorhynchus	*Mercenaria
*Salmo	*Crassostrea
*Crassostrea	Aequipecten
*Ostrea	Callinectes
Pelecypoda	Cancer
Haliotis	
Homarus	Gulf States
Pandalus	*Penaeus
Cancer	*Macrobrachium
Morone	*Trachinotus
Platichthys	*Procambarus
Engraulis	Morone
Marine Algae	Mugil
	Salmo
Northeast	Epinephalus
*Mercenaria	Micropogon
*Crassostrea	Centropristis
*Ostrea	Marine Algae
Mya	Crassostrea
Placopecten	Mercenaria
Homarus	
	Hawaii
Caribbean	Chelonia
*Chelonia	Mugil
*Penaeus	Coryphaena
*Macrobrachium	Chanos
Crassostrea	Macrobrachium

Table 4. Survey rank of acceptability of agencies to conduct quarantine inspection of aquaculture products

Agency	Rank Rating
National Marine Fisheries Service	2.25
Bureau of Sportfish and Wildlife	2.68
U. S. Department of Agriculture	2.91
State Fish and Game	2.96
State University	4.18
New Federal Agency	5.25
U. S. Customs	5.84
Private Consultant	6.22

Table 5. Hatchery record for penaeid shrimp at Turkey Point (1969 to 1974)

Total Runs: 33
Problem Runs: 19

Reason for Problem	Number of Runs
Biological Failure	7
Disease	2
Human Error	4
Equipment Failure	2
Combination (Human Error and Biological and Equipment Failure)	3

Table 6. Frequency of survival of penaeid shrimp in pond culture studies at Turkey Point, Florida

Percent Survival	Winter	Summer
0- 9	8	3
10-19	0	1
20-29	1	2
30-39	2	0
40-49	7	0
50-59	1	6
60-69	0	0
70-79	1	6
80-89	2	5
90-96	1	3
Total Ponds	23	26

AN OVERVIEW OF AQUATIC ANIMAL DISEASE RESEARCH AT THE GULF COAST RESEARCH LABORATORY

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Since its organization in 1947, the Gulf Coast Research Laboratory has been concerned with all areas of research relating to aquatic marine animals, but until the late 1960's no concerted effort was made to study the parasites and diseases of these animals. In 1969, the Gulf Coast Research Laboratory received a Federal Aid to the Fisheries grant from the National Marine Fisheries Service to initiate studies on the parasites and diseases of marine animals with potential for mariculture. To date grant support has continued from this source and with additional research support from the Sea Grant Program and the State of Mississippi, the overall parasite and disease research program has expanded.

The following is an annotated bibliography of papers originating from the Gulf Coast Research Laboratory and relating to studies of parasites and diseases of aquatic animals.

1969

Christmas, J. Y. 1969. Parasitic barnacles in Mississippi estuaries with special reference to Loxothylacus texanus Boschma in the blue crab (Callinectes sapidus). Proceedings of the Twenty-Second Annual Conference. Southeastern Association of Game and Fish Comissioners. pp. 272-275.

Specimens of Callinectes sapidus, infected with Loxothylacus texanus were collected from the Mississippi Sound. Sacculinid infections were not observed in Callinectes similis although infected speimens of Eurypanopeus depressus and Rhithropanopeus harrissii were encountered.

1970

Christmas, J. Y., and H. D. Howse. 1970. The occurrence of lymphocystis in Micropogon undulatus and Cynoscion arenarius from Mississippi estuaries. Gulf Research Reports, 3(1):131-154.

Lymphocystis was observed in Atlantic croakers (Micropogon undulatus) and sand seatrouts (Cynoscion arenarius) collected from brackish waters of the Mississippi Gulf Coast. This is the first report of lymphocystis in fishes of the Gulf of Mexico and adds one family and two species to host records.

Howse, Harold D., and James Y. Christmas. 1970. Lymphocystis tumors: Histochemical identification of hyaline substances. Transactions of American Microscopical Society 89(2): 276-282.

Hyaline substances, capsules, and matrices, of lymphocystis tumors in the Atlantic croaker, Micropogon undulatus (Linnaeus), and the sand seatrout, Cynoscion arenarius, were studied histochemically and found to be composed of acid mucopolysaccharides that contain functional sulfate groups.

Overstreet, Robin M. 1970. Spinitectus beaveri sp. n. (Nematoda: Spiruroidea) from the bonefish, Albula vulpes (Linnaeus), in Florida. The Journal of Parasitology 56(1): 128-130.

Spinitectus beaveri sp. n. from the stomach of Albula vulpes is described.

Overstreet, Robin M. 1970. Baylisascaris procyonis (Stefanski and Zarnowski, 1951) from the kinkajou, Potos flavus, in Columbia. Proceedings of the Helminthological Society of Washington. 37(2): 192-195.

Specimens of Baylisascaris procyonis are described from the kinkajou, Potos flavus, and compared with others from the raccoon, Procyon lotor, from California. This new host record extends the geographical range of the worm into South America.

Overstreet, Robin M. 1970. A syncoeliid (Hemiuroidea Faust, 1929) metacercaria on a copepod from the Atlantic equatorial current. The Journal of Parasitology 56(4): 834-836.

A species of Syncoelium parasitizing the copepod Candacia pachydatyla is discussed.

Overstreet, Robin M., and Charles E. Brown. 1970. Lasiotocus trachinoti sp. n. (Digenea: Monorchiidae) from the pompano, Trachinotus carolinus (Linnaeus), along the east coast of Florida. The Journal of Parasitology 56(5): 941-943.

The monorchiid trematode Lasiotocus trachinoti sp. n. is described from the intestine and pyloric ceca of young Trachinotus carolinus. It is most like L. mulli of Bartoli and Prevot, 1966, but differs from it primarily by being smaller and having cirrus spines more evenly dispersed.

Overstreet, Robin M. 1970. Two new species of digenea from the spot, Leiostomus xanthurus Lacepede, from the Gulf of Mexico. The Journal of Parasitology 56(6): 1055-1057.

Two new species of digenea (Apocreadium manteri and Lecithaster leiostomi) are described from the euryhaline fish, Leiostomus xanthurus, from near Ocean Springs, Mississippi.

1971

Howse, H. D., and J. Y. Christmas. 1971. Observations on the ultrastructure of lymphocystis virus in the Atlantic croaker, Micropogon undulatus (Linneaus). Virology, 44(1): 211-214.

The virus particles are osmiophilic and are scattered throughout the cytoplasm. They are not present in the nucleus. The particles are polyhedral and range from 240 to 260 nm in diameter.

Overstreet, Robin M. 1971. Neochasmus sogandaresi sp. n. (Trematoda:Cryptogonimidae) from the striped bass in Mississippi. Transactions of American Microscopical Society 90(1): 87-89.

The cryptogonimid trematode Neochasmus sogandaresi sp. n. is described from the intestine and pyloric caeca of a single specimen of the striped bass Morone saxatilis (Walbaum, 1972) from the West Pascagoula River in Mississippi.

Overstreet, Robin M. 1971. Metadena spectanda Travassos, Freitas, and Buhrnheim, 1967, (Digenea: Cryptogonimidae) in estuarine fishes from the Gulf of Mexico. Proceedings of the Helminthological Society of Washington, 38(2): 156-158.

The first report of Metadena spectanda Travassos, Freitas, and Buhrnheim, 1967, from North American waters is given. The cryptogonimid trematode was found in Micropogon undulatus and Bairdiella chrysura and redescribed from specimens from the former.

Overstreet, Robin M. 1971. Glaucivermis spinosus gen. et sp. n. (Digenea: Zoogonidae) from the southern kingfish, Menticirrhus americanus (Linnaeus), in the coastal waters of Mississippi. The Journal of Parasitology, 57(3): 536-538.

Glaucivermis spinosus is described from the intestine and pyloric ceca of Menticirrhus americanus collected near Ocean Springs, Mississippi, in the Gulf of Mexico and adjacnet waters.

Overstreet, Robin M. 1971. Some adult digenetic trematodes in striped mullet from the northern Gulf of Mexico. The Journal of Parasitology, 57(5): 967-974.

Morphological information is given on the adults of four trematodes from Mugil cephalus from estuarine waters of the nothern Gulf of Mexico with complementary information from specimens from Georgia.

1972

Cook, David W. 1972. A circulating seawater system for experimental studies with crabs. The Progressive Fish-Culturist, 34(1): 61-62.

This system, which was designed for simplicity of construction, low cost, and minimum maintenance, is applicable for holding crabs and other aquatic animals for disease studies.

Cook, David W. 1972. Experimental infection studies with lymphocystis from Atlantic croaker. Proceedings of the Third Annual Workshop World Mariculture Society, pp. 329-335.

Aquarium studies were employed to elucidate the mode of transmission and progression of lymphocystis disease in croaker (Micropogon undulatus). In host range studies, only members of the family Sciaenidae could be infected with the virus from the croaker.

Howse, H. D. 1972. Snook (Centropomus: Centropomidae): New host for lymphocystis, including observations on the ultrastructure of the virus. The American Midland Naturalist, 88(2): 476-478.

Lymphocystis disease is reported in a new host species and family (Centropomus undecimalis (Bloch): Centropomidae). The virions are icosahedral in shape and range from 237 to 250 nm in diameter. Each has a necleoid of about 180-190 nm in diam consisting of a ball of osmiophilic threads having cross-sectional diameter of about 3 nm.

Overstreet, Robin M., and David W. Cook. 1972. An underexploited Gulf Coast fishery, soft shelled crabbing. The American Fish Farmer, 3(9): 12-17.

This semi-technical article describes the problems and potential of the soft shell crab industry in the northern Gulf of Mexico.

Overstreet, Robin M., and Harriet M. Perry. 1972. A new microphallid trematode from the blue crab in the northern Gulf of Mexico. Transactions of American Microscopical Society 91(3): 436-440.

Levinseniella (Monarrhenos) capitanea n. sp. is described from metacercariae on the hepatopancreas and gonads of Callinectes sapidus Rathbun from Louisiana and Mississippi.

Zwerner, D. E., and A. R. Lawler. 1972. Some parasites of Chesapeake Bay fauna. pp. 78-94. In Wass, M. L. (editor). A check list of the biota of lower Chesapeake Bay. Va. Inst. Mar. Sci., Spec. Sci. Rept. No. 65, 290 p.

A checklist of parasites found in the lower Chesapeake Bay is presented.

Lawler, Adrian R., and Boris Iv. Lebedev. 1972. Encotyllabe latridis Lebedev, 1967 and Mediavagina forsteri Lawler and Hargis, 1968 are identical Monogeneans. Proceedings of The Helminthological Society of Washington, 39(2): 267-268.

The paper proposes the transfer of Encotyllable latridis to the genus Mediavagina Lawler and Hargis, 1968.

Overstreet, R. M. 1972. Digenetic trematodes of the the Chesapeake Bay. Chesapeake Science, Vol. 13, Supplement: 106-107.

The article describes our present state of knowledge concerning the digenetic trematodes of the Chesapeake Bay.

1973

Dailey, Murray D., and Robin M. Overstreet. 1973. Cathetocephalus thatcheri gen. et sp. n. (Tetraphyllidea: Cathetocephalidae fam. n.) from the bull shark: a species demonstrating multistrobilization. Journal of Parasitology, 59(3): 469-473.

Cathetocephalus thatcheri, a multistrobilate tapeworm, is described from the spiral valve of the bull shark, Carcharhinus leucas (Valenciennes), taken in the Gulf of Mexico and the Atlantic coast of Florida. A new family is created for the new genus.

Cook, David W., and Sandra R. Lofton. 1973. Chitinoclastic bacteria associated with shell disease in Penaeus shrimp and the blue crab (Callinectes sapidus). Journal of Wildlife Diseases, 9: 154-159.

The occurrance of shell disease in three species of penaeid shrimp is reported. Chitinoclastic bacteria isolated from lesions on these shrimp and from lesions on the blue crab were classified as members of the genera Beneckea, Vibrio, and Pseudomonas. One type of Beneckea was present in all cases of shell disease encountered, making this organism suspect of being the causative agent

Overstreet, Robin M. 1973. Parasites of some penaeid shrimps with emphasis on reared hosts. Aquaculture, 2: 105-140.

Information is presented about parasites and commensals of penaeid shrimps, allowing those who rear shrimp and those who are interested in associated organisms of shrimp to have a better knowledge of the organisms that are or may be associated with brown, white, and pink shrimp.

Overstreet, Robin M. 1973. Some species of Lecithaster Luhe, 1901 (Digenea: Hemiuridae) and related genera from fishes in the northern Gulf of Mexico. Transactions of American Microscopical Society 92(2): 231-240.

Lecithaster helodes n. sp. is described from Mugil curema and M. cephalus. New host records are listed for several other hemiurid trematodes.

Lawler, Adrian R. 1973. A non-baited fish trap for shallow water. The Progressive Fish-Culturist, 35(4): 237-238.

The article describes a simple trap used to collect fish for parasite and disease studdes.

Nickol, Brent B., and Richard W. Heard, III. 1973. Host-parasite relationships of Fessisentis necturorum (Acanthocephala: Fessisentidae). Proceedings of the Helminthological Society of Washington, 40(2): 204-208.

In northeastern Georgia, larvae of Ambystoma opacum and Pseudotriton montanus are heavily parasitized by F. necturorum. In this region larval salamanders begin acquiring infections of F. necturorum during winter months and parasite prevalence increases to a peak in early spring. When metamorphosis begins in late April, acanthocephalan infections decline until they are apparently absent in fully metamorphosed A. opacum and Pseudotriton montanus.

Lawler, A. R., and T. W. Dukes. 1973. Lymphocystis in the eye. The Veterinary Record, 93(10): 297.

The article notes lymphocystis infection in the eye of the sand seatrout.

Lom, Jiri, and Adrian R. Lawler. 1973. An ultrastructural study on the mode of attachment in dinoflagellates invading gills of cyprinodontidae. Protistologica, 9: 293-309.

The ultrastructure of two ectoparasitic dinoflagellates Oodinium cyprinodontum Lawler, 1967 and Amyloodinium sp. was studied with respect to their mode of attachment.

1974

Overstreet, Robin M., and Douglas M. Martin. 1974. Some digenetic trematodes from synaphobranchid eels. The Journal of Parasitology, 60(1): 80-84.

Four trematodes, all new host records, were found in synaphobranchid eels from continental slopes in the Gulf of Mexico and from the Straits of Florida.

Lawler, A. R., H. D. Howse, and D. W. Cook. 1974. Silver perch, Bairdiella chrysura: New host for lymphocystis. Copeia, 1974 (1): 266-269.

Spontaneous lymphocystis lesions were found both externally and internally. Internal infections were found in the spleen, mesenteries, liver, heart, kidneys, ovaries, testes, and gall bladder.

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