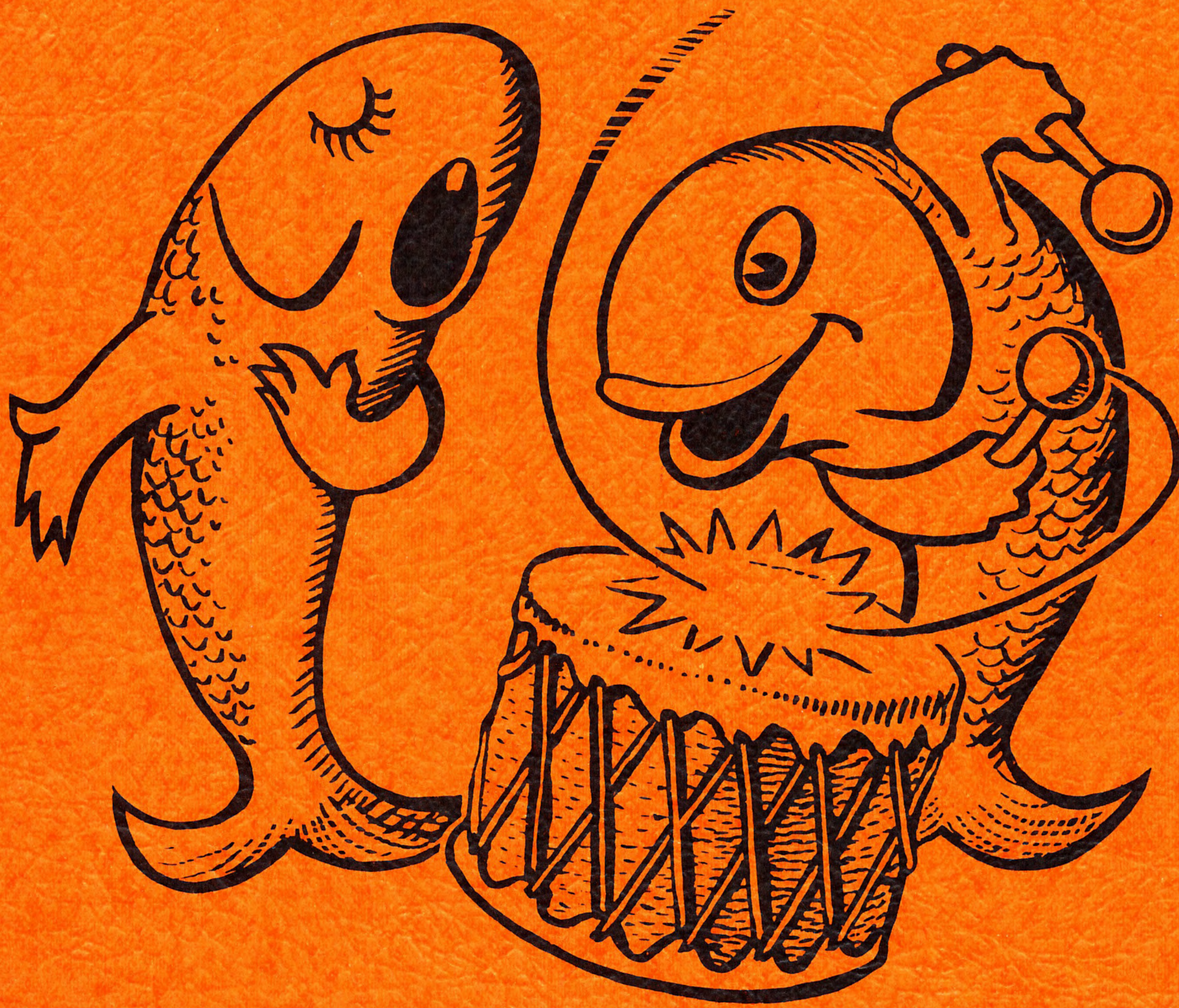
The DRUM and CROAKER

A Highly Irregular Journal for the Public Aquarist



MARCH 1976

VOLUME 16 (76), NUMBER 1

VOLUME 16 (76), NUMBER 1 MARCH 1976

D R U M A N D C R O A K E R

The Informal Organ

for

Aquarists

Manuscript material received is reproduced with a minimum of editing and is not returned unless requested.

We take credit for neither spelling, nor classification.

This issue of DRUM AND CROAKER compiled by Kim J. Marggraf, former Secretary to the Director, John G. Shedd Aquarium.

Prepared by the John G. Shedd Aquarium; 1200 South Lake Shore Drive; Chicago, Illinois as a service to aquariums generally.

NOTICE

With this issue the John G. Shedd Aquarium will turn over the responsibility for editing, publishing and distributing to the New England Aquarium. John Prescott has agreed to do the honors for a few years.

We are proud to have had a part in the publishing history of this unique, elite and sacred journal. Kim Marggraf, Editor of Drum and Croaker, and my Secretary, has left Shedd Aquarium for other pursuits. She deserves our thanks for the countless hours she spent typing, assembling, and mailing these words of wisdom.

Kim Marggraf
Adios Amigos

All future articles, inquiries and complaints should be addressed to:

Editor, Drum and Croaker
New England Aquarium
Central Wharf
Boston, Massachusetts 02110

William P. Braker
Director
John G. Shedd Aquarium

CONTENTS

	PAGE
REPRODUCTION AND POTENTIAL RESEARCH USE OF THE GYNOGENETIC TELEOST POECILLA FORMOSA R.W. Hart, H.R. Livesey and R.B. Setlow	1
MIAMI SEAQUARIUM'S LEATHERBACK TURTLE Pat Foster	11
INVESTIGATION OF SODIUM CHLORITE FOR USE IN MARINE SYSTEMS Bruce M. Poole	14
AN OVERVIEW OF MYSTIC MARINELIFE AQUARIUM	23
THOUGHTS ON PLANNING A ZOO AQUARIUM U. Erich Friese and Ronald Strahan	26
NOTES ON SOME PARASITES OF BAHAMA FISHES Harvey D. Blankespoor	35
SKIN INFECTIONS CAUSED BY INOCULATION OF MYCOBACTERIUM MARINUM FROM AQUARIUMS J. Walter Wilson, M.D.	39
A NEW AERATION SYSTEM DEVELOPED BY THE WAIKIKI AQUARIUM STAFF Frank Sutherland	43
AMMONIUM CHLORIDE AND BIOLOGICAL FILTRATION Stephen D. Walker	46
FISH FANCIERS CROSSWORD	49

ILLUSTRATIONS

PTEROPOD: CLIONE SP. "FLYING SNAIL" Charles Eames	45

REPRODUCTION AND POTENTIAL RESEARCH USE
OF THE GYNOGENETIC TELEOST POECILLA FORMOSA*

R. W. Hart[1,2], H. R. Livesey[1], and R. B. Setlow[1]

Abstract

Poecilia formosa, a gynogenetic teleost being used as a research tool, has been studied as to its care, physiology, and methods of reproduction. The time between spawns and spawn size are dependent upon both temperature and season. The frequency of sexual reproduction, as indicated by a color marker, is dependent on spawn size, clone, and season. Transplantation experiments can be used to determine genetic similarity between clone and hybrids. In two cases a third form of reproduction which is entirely independent of males has been noted. Occurrence of this form of reproduction is less than 0.003%.

Introduction

Poecilia formosa (Girard) seemingly arose from hybridization of *P. latipinne* (Lesueur) and *P. mexicana* (Steindachner) (1). This species is one of the naturally occurring examples in the fishes of gynogensis (2) (activation of unfertilized eggs by sperm without subsequent genetic contribution). Schultz (3) has recently reviewed the evidence for gynogenesis in other fishes, including *Poeciliopsis* and *Carassius*. Previous work indicated that inheritance is maternal and the populations in nature exist as histocompatible clones (4). It was reported after a study of 8000 "breedings" that *P. formosa* was gynogenetic (5), although the occasional occurrence of a male in the wild population was an enigma (6). Hubbs (7) has since reported the occurrence of several other males in the wild and has suggested the possibility of sexual reproduction. Recent studies have

---

*Research supported by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation and by the National Cancer Institute. R. W. Hart was a postdoctoral fellow of the National Cancer Institute.

[1]Carcinogenes is Program, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 37830

[2]Department of Radiology, The Ohio State University Hospitals, Columbus, Ohio, 43210

demonstrated that triploid offspring occur in field populations of P. formosa and P. mexicana, thus supporting the assumption of sexual reproduction (8). The first report of sexual reproduction within P. formosa (9) was strongly questioned, but the occurrence of sexual reproduction has since been supported by subsequent observations by Kallman (10), Rasch et al. (11), Schultz and Kallman (12), and Prehn and Rasch (13). Due to the cost of maintenance of large numbers of fish and the time needed to perform experiments, few studies have been carried out on the frequency of the various methods of reproduction, the normal rates of aging within the species, or the optimal methods for care. This particular species has recently been used in research projects in cancer (14), genetics (15-17), and evolution (2, 18). For this reason it was of importance that the basic parameters for handling and breeding of P. formosa be explored.

Materials and Methods

All clones were originally collected from a freshwater pool near Brownsville, Texas, by C. P. Haskins in 1946 and have been kept under greenhouse conditions since that time (19). They were classified as to histologically compatible types by Kallman in 1962 and separated on this basis into four separate clones (20). C. P. Haskins generously supplied us with samples of these clones in 1971. Several breeders were raised in individual 10-gallon tanks containing a single male (Gambusia yucatana) (21). Resulting spawns were immediately removed, counted, and placed in 29-gallon tanks at a density of about 2-3 fish per gallon. After 2 to 3 months these individuals were removed and placed in 125-gallon tanks at a density of about 1 fish per gallon. Fish were counted at each transfer and routinely screened for disease or death twice daily. Water temperature was maintained at 24± 2°C with a photoperiod of 16 hr light and 8 hr dark. Filtration was maintained by a combination of undergravel filters and bottom filters; additional filtration when needed was performed with a diatomaceous earth filter. All tanks were maintained under isolation conditions with complete separation of nets, water, filters, and plants, etc. Twenty-five percent of the water in each tank was drained and replaced with aged water weekly. Every 2 months tanks were drained down and cleaned, old gravel was replaced with fresh washed gravel, and charcoal filters were changed.

All fish were fed twice daily with live baby brine shrimp and with a high-protein porridge-type food.[3] We originally used flake food but changed because of its high cost and the slow growth of fish. The fish are fed only sufficient food to be consumed in 10 min, so as to minimize bacterial growth in the tank.

[3]Available from Harry's Aquarium, Oak Ridge, Tennessee.

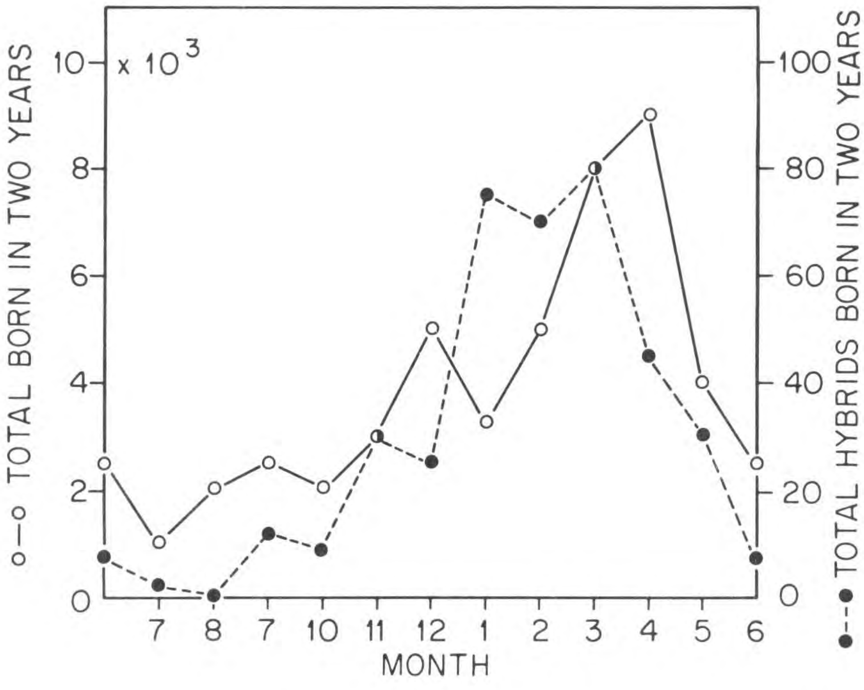


Figure 1. Fish born in two years as a function of month of birth: solid line indicates total births; segmented line indicates hybrid births

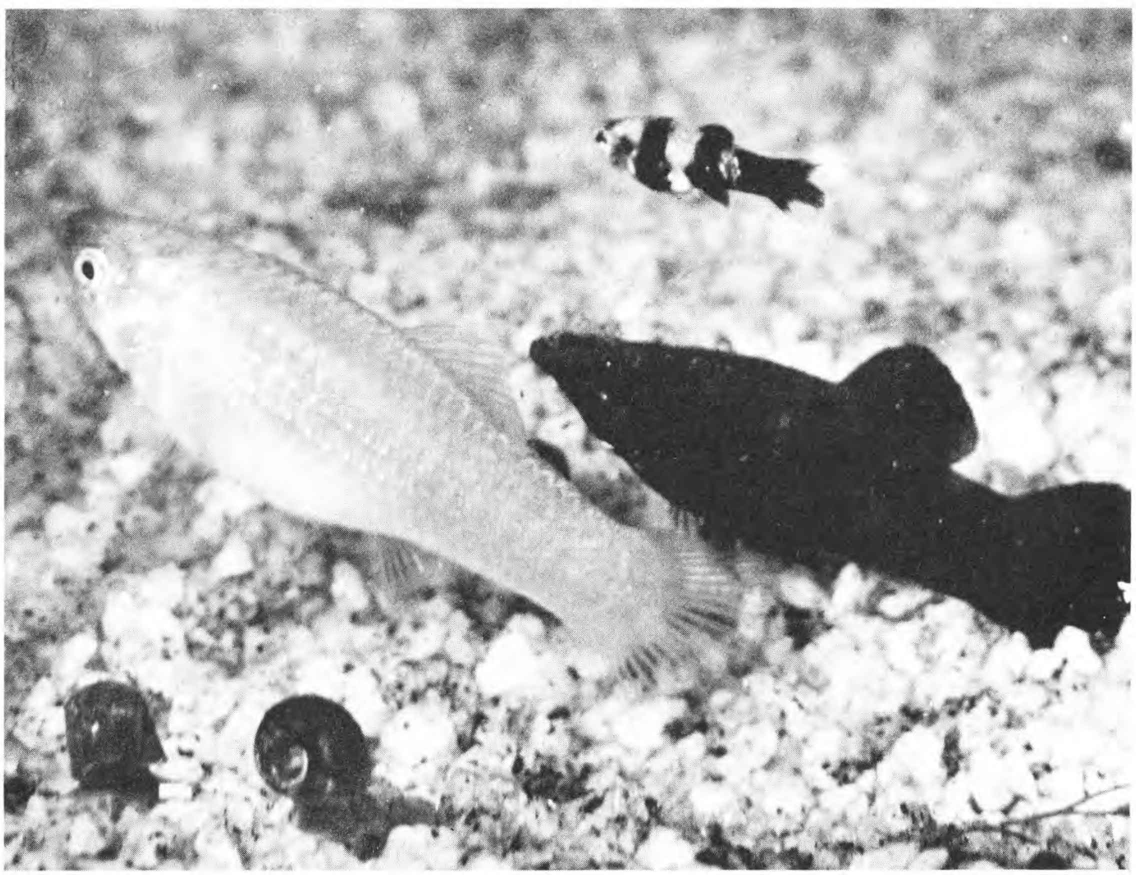


Figure 2. P. formosa female (white), G. yucatana male (black), and hybrid offspring (striped).

Results and Discussion

I. Modes of Reproduction. When females of P. formosa (white) were mated to males of G. yucatana (black), both white and various colored offspring were produced.

A. Non-sexual reproduction. The white fish represent 99% of the offspring and, by histocompatibility tests (see below), are identical to the female parent. This nonsexual reproduction is the standard mode of reproduction within the species. As is illustrated in Fig. 1, nonsexual reproduction occurs year round. The size of spawn varies with the season, as does the time between spawns. These variations may represent a wavelength-dependent intrinsic annual rhythm, since we attempted to keep all growth conditions constant (e.g., temperature, feeding time, tank volume, photoperiod, etc.) except for supplemental natural light which obviously varied with the season. It is interesting to note here that peak production for the species occurs at the time of peak rainfall and food supply within the area of origin. Since their method of nonsexual reproduction has recently been reviewed by Schultz (18), along with its possible evolutionary significance and adoptive advantages and disadvantages, it will only be briefly discussed here.

Asexual reproduction within the vertebrates occurs primarily within the teleosts, the two most common forms being gynogenesis and hybridogenesis. In gynogenesis it is generally assumed that the sperm stimulates the ovum of P. formosa to develop without pronuclear fusion (22). The offspring are all female and have no male phenotypic characteristics. They are diploid and must achieve diploidy by either (a) reentry of a polar body or (b) doubling of the premeiotic chromosome without a nuclear division (23, 24, 25).

Hybridogenesis also depends on sperm from bisexual host species. However, gametic fusion occurs between the haploid pronuclei, and the resulting diploid progeny have traits from both parents. The difference between true hybridization and hybridogenesis is that in the latter random segregation does not occur, and in each generation only the maternal genes are inherited and the paternal are lost. Hybridogenesis has not been reported in P. formosa; however, it has been well established in its relative species Poeciliopsis (2).

A third form of asexual reproduction which occurs within the teleosts has been termed spontaneous parthenogenesis. These cases are extremely rare, and due to the ability of many teleosts to retain sperm for a long period of time each case must be verified very carefully (2). This form of reproduction has been noted in our laboratory and is here termed non-male-dependent gynogenetic reproduction. The offspring produced in our breeder tanks are immediately removed and placed in 29-gallon tanks, where they are held for periods of 2 to 6 months prior to experimentation. These individuals are in the presence of males for a maximum of only 2 days after birth. In one case, with a sample of approximately 100 fish derived from Clone 2, the fish were maintained for approximately 1 year without use. During this time two of the fish produced young. The fish within that tank were examined for the possible presence of gynopodia but none were found. Since they were removed at an age of less than 2 days from the breeder tanks, either exceptionally young fish are capable of storage of sperm for exceedingly long periods of time, or non-male-dependent gynogenetic reproduction is possible. The young were examined in all cases and found to be diploid, by use of DNA cytophotometry (17), thereby leaving open the possibility that gynogenetic reproduction can be stimulated by factors other than male sperm. This observation has since been repeated. As far as we can find this is the only case reported of non-male-dependent gynogenetic reproduction within P. formosa. The mechanism by which such a phenomenon could occur is not readily understandable, but its possible evolutionary significance is of interest.

B. Sexual reproduction. Figure 2 shows a hybrid resulting from a cross between P. formosa and G. yucatana. The hybrid offspring can easily be identified by the presence of various color patterns. The number of dissimilar offspring was dependent upon the clone studied. Of the over 50,000 offspring in the three clones observed in our laboratory (Haskins' classification Clones 2, 3, and 4), two clones yielded phenotypically different offspring (Clone 4, 1.0%; Clone 2, 0.65%). The distribution was nonrandom, with only 12 breeder animals out of 700 accounting for all hybrids in both clones. Seventy-five percent of the hybrids occurred when spawn size was 70 or greater and primarily during the months of December through May (see Fig. 1), though clones of this size or greater occurred only 20% of the time during these months.

C. Histocompatibility tests. In a gynogenetic species there is immunological homogeneity between mother and offspring (20). One method to determine the extent of immunological interrelationship between individuals is by a tissue transplantation test. Transplantation tests were conducted between phenotypically similar and dissimilar offspring of P. formosa, as well as between P. formosa and G. yucatana. Hearts, spleens, and anal, dorsal, and caudal fins were transplanted according to the method of Kallman and Gordon (26). When the donor was retained for subsequent transplants only the dorsal fin was removed. Infection after transplant was minimized by postsurgical restraint in an 8.6% sea salt solution. The results of these experiments, shown in Table 1, indicate three things. (a) P. formosa reproduces by genogensis and the differences between clones has been retained over the 25 years of their laboratory maintenance. (Intraclone transplants are not rejected, but interclone or interspecies ones are.) (b) The color marker is a good indication of sexual reproduction, and the hybrids show some histocompatibility for both parents. [Transplantation of tissue from the clone of origin into the hybrid leads to rejection in all cases within a period of 45 days or less, where as interclone transplantations were rejected within 18 days (parts B, D). Transplants between G. yucatana and clones of P. formosa are rejected sooner than between G. yucatana and hybrids (parts C, F). Moreover, transplants between hybrid animals of the same clonal origin survive longer than do ones between different clones (part 3).] (c) The hybrid color mosaics are also immunological mosaics. The rejection time for hybrid dermis transplanted into the clone of origin depends on whether the dermis is black or white (part G). White dermis is not rejected at all and black dermis is rejected slower than interclone or interspecies transplants.

Table 1. Rejection times, ± standard deviations, for transplants among clones of P. formosa and P. yucatan and their hybrid offspring. Transplants are identified by clone numbers.

Transplant	Organ	No. of transplants	Avg. rejection time (days)
A. Intraclone			
2 → 2	Anal fin	26	>150
2 → 2	Spleen	20	>150
2 → 2	Heart	20	>150
B. Interclone			
2 → 4	Anal fin	26	18 ± 3
4 → 2	Anal fin	26	17 ± 4
C. Interspecies			
4 → G. yucatan	Anal fin	15	8 ± 3
2 → G. yucatan	Anal fin	15	7 ± 1
G. yucatan → 4	Anal fin	15	9 ± 4
G. yucatan → 2	Anal fin	15	8 ± 3
D. Clone → hybrid			
2 → 2	Heart	3	41 ± 10
2 → 2	Spleen	3	41 ± 5
2 → 2	Anal fin	3	43 ± 12
2 → 2	Dorsal fin	3	44 ± 3
2 → 2	Caudal fin	3	44 ± 9
4 → 4	Heart	3	42 ± 12
4 → 4	Spleen	3	40 ± 1
4 → 4	Anal fin	3	45 ± 8
4 → 4	Dorsal fin	3	34 ± 14
4 → 4	Caudal fin	3	45 ± 9
4 → 4	Anal fin	5	33 ± 2
4 → 4	Caudal fin	5	33 ± 13
E. Interhybrid			
4 → 2	Anal fin	10	12 ± 2
2 → 4	Anal fin	10	11 ± 2
4 → 4	Anal fin	5	34 ± 15
2 → 2	Anal fin	5	29 ± 12
F. G. yucatan → hybrid			
G. yucatan → 2	Heart	3	16 ± 5
G. yucatan → 2	Dermis	3	14 ± 5
G. yucatan → 4	Heart	3	19 ± 3
G. yucatan → 4	Spleen	3	19 ± 3
G. Hybrid → Clone			
2 → 2	Dark dermis	10	25 ± 8
2 → 4	Dark dermis	10	12 ± 4
4 → 2	Dark dermis	10	13 ± 1
4 → 4	Dark dermis	10	23 ± 5
2 → 2	Light dermis	10	>150
2 → 4	Light dermis	10	18 ± 3
4 → 2	Light dermis	10	15 ± 3
4 → 4	Light dermis	10	>150

Table 2. Disease and drug effectiveness for P. formosa

	Mycobacterium		Fungus	Protozoan
	In vivo	In vitro		
Isoniazid (µg/g)				
0.01	--	+	-	-
0.1	++	+++	-	-
1.0	+++	+++	-	-
Penicillin (units/Kg)				
10	-	+	-	-
50	+	+	-	-
Streptomycin (µg/g)				
0.001	-	-	-	-
0.01	++	-	-	-
0.02	++	+	-	-
Maracyn				
20 mg/gal	-	-	++	-
Tetracycline				
20 mg/gal	-	-	+++	+
$CuSO_4$ (1.0%)				
+ Formalin (1.0%)	-	-	-	++
5 ml/gal				
Gentamycin $\cdot SO_4$ (µg/g)				
10	-	+	-	-
20	+	++	-	-

D. Biological properties of hybrids. The number of fertile offspring is only 10%. Spawn size is normal, and the occurrence of noncolored marked individuals predominates. (Presumably most of the offspring of hybrids are gynogenetically derived.) Although we have maintained fewer than 100 hybrids for extended time periods, there have been two spontaneous tumors in this group, whereas we have observed only this number in over 10,000 nonhybrids.

II. Disease: Occurrence and Treatment. Of primary importance to the development of any species for laboratory use is a knowledge of the frequency and cause of death. We have analyzed either by gross pathology or histopathology the cause of death over a 2-year period within approximately 100 of our tanks (27). By far the most frequent cause of death (75%) in our nonexperimental animals is infection by Mycobacterium fortuitum. The lesions are characteristically large reddish-blue granulomas, staining positively for Mycobacterium sp. Approximately 15% of fish die from fungal infection observed as a white fluffy growth, primarily on the caudal fin. If left untreated the infection will spread over the entire animal and lead to dermal degradation and secondary infection by bacterial agents. The remaining 10% die from a wide spectrum of causes, ranging from an occasional protozoan invasion to various forms of bacterial attack. In only 2% of the cases has there been no apparent cause of death and in only one case out of several thousand has there been a confirmed occurrence of cancer. In the latter case the tumor was diagnosed as a melanoma.

Table 2 summarizes the various treatments that have been attempted and analyzed for effectiveness with respect to these various diseases. With respect to Mycobacterium, no treatment seems to effect a cure. However, isoniazid at a concentration of 0.70 mg/ml acts as a prophylactic and restrains the growth of lesions but will not reverse the disease, nor will an intraperitoneal (IP) infection of 1 μg/g body weight. A sensitivity test with a broad spectrum of antibiotics showed that the bacteria were sensitive both to isoniazid and high concentrations of gentamycin $\cdot$ SO_4 but were refractory to the other antibiotics shown in Table 2. To test whether isoniazid at 1 μg/g tissue would act as a prophylactic, we took a random sample of 300 fish and divided them into two groups of 150 each. In the control group (age 4-10 months), over a 10-month period, 65% of the fish died of Mycobacterium lesions. In the group injected with isoniazid at a concentration, only 12% developed lesions and only 2% died of them. Thus isoniazid acts as a prophylactic, and even though Mycobacterium is generally assumed to be in the cyst form in the animal, the drug seems to prevent expression over an extended period of time (27), although it is not a useful method of treating large numbers of fish.

In the case of the predominant fungal infection, which we have termed white disease, Maracyn (Mardel Laboratories, Inc.) at a concentration of 20 mg/gallon applied daily for 4 days seems to be effective in elimination of this disease. Tetracycline at the same concentration is also effective, but because of its toxicity 25% of the water must be changed each day. An added advantage of treatment with Maracyn seems to be its wide-range effect on several other bacterial infections and thus its ability to prevent subsequent infections.

III. Potential Research Use of Gynogenetic Teleosts. A major difficulty in whole-animal research has always been the extreme range of variability that occurs within a given species. Even in the case of inbred mouse strains there still exists a range of response to many agents. It is highly desirable, therefore, to develop any research tool which minimizes this range of variability. The teleost P. formosa seems ideal. Due to its nonsexual mode of reproduction the offspring of any given fish should be identical to each other and and to any member of that clone. Our histocompatibility tests indeed support this conclusion. Thus one may increase the resolution of an experiment in which the variation between members of control and experimental series would be detrimental. Such experiments might involve (a) circadian rhythms in which individual variability induced by slight genetic difference might lead to alterations in the free running rhythm; (b) the isolation of individual mutants, expecially when trying to determine the effects of certain environmental mutagens on whole animals, since production of a single morphological modification can be readily identified and analyzed (Note that, since these are aquatic animals, one can test environmental mutagens soluble in the aqueous environment.); and (c) the testing of carcinogens either by dissolving them in the aqueous environment of the fish or by treating cells in vitro and subsequently injecting them into the (histocompatible) members of the same clone so as to test for malignant transformation.

From an evolutionary standpoint the development of gynogenesis by itself is an interesting problem; however, beyond this lies the possible development of a tool that can be utilized for understanding the rate of spontaneous mutation induction, its frequency of occurrence, and the role it plays in species differentiation.

References

1. P. Abramoff, R. M. Darnell, J. S. Balsano, Amer. Natur. 102, 555(1968).

2. R. J. Schultz, Am. Zoologist, 11, 351(1971).

3. R. J. Schultz, Amer. Natur. 103, 605(1969).

4. K. D. Kallman, in XVI International Congress of Zoology, Proceedings, J. A. Moore, Ed. (XVI Int. Congr. Zool., Washington, D.C., 1963), vol. 2, p. 170; R. M. Darnell and P. Abramoff, Copeia, 354(1968).

5. C. L. Hubbs and L. C. Hubbs, Aquar. J. 17, 3(1946).

6. C. L. Hubbs, G. E. Dewy, B. Warburton, Science 129, 1227(1959).

7. C. L. Hubbs, Bull. Texas Memorial Mus., No. 8, 317(1964),

8. L. M. Prehn and E. M. Rasch, Can. J. Genet. Cytol. 11, 880(1969).

9. C. P. Haskins, E. F. Haskins, R. E. Hewitt, Evolution 14, 473(1960).

10. K. D. Kallman and R. W. Harrington, Jr., Biol. Bull. 126, 101(1964).

11. E. M. Rasch, R. M. Darnell, K. D. Kallman, P. Abramoff, J. Exp. Zool. 160, 155(1965).

12. R. J. Schultz and K. D. Kallman, Nature 219, 280(1968).

13. L. M. Prehn and E. M. Rasch, Can. J. Genet. Cytol. 11, 880(1969).

14. R. W. Hart and R. B. Setlow, Abs. 1st Annual Meeting of the Am. Soc. for Photobiol., Sarasota, Fla., 120(1973).

15. K. D. Kallman, Transplantation Proc. 2, 263(1970).

16. K. D. Kallman, Genetics 50, 260(1968).

17. E. M. Rasch, L. M. Prehn, R. W. Rasch, Chromosoma 31, 18(1970).

18. R. J. Schultz and K. D. Kallman, Nature 219, 280(1968).

19. Personal communication with C. P. Haskins.

20. K. D. Kallman, J. Genet. 58, 7(1962).

21. G. Sterba, in Freshwater Fishes of the World. Pet Library Ltd., New York, 564(1966).

22. C. L. Hubbs and L. C. Hubbs, Science 76, 628(1932).

23. H. J. Meyer, Genetics 36, 22(1938)

24. H. C. MacGregor and J. M. Uzzel, Jr., Science 143, 1043(1964).

25. A. R. Sanderson, Proc. Roy. Soc. Edin. 67, 333(1960).

26. K. D. Kallman and M. Gordon, Ann. N. Y. Acad. Sci. 71,305(1957).

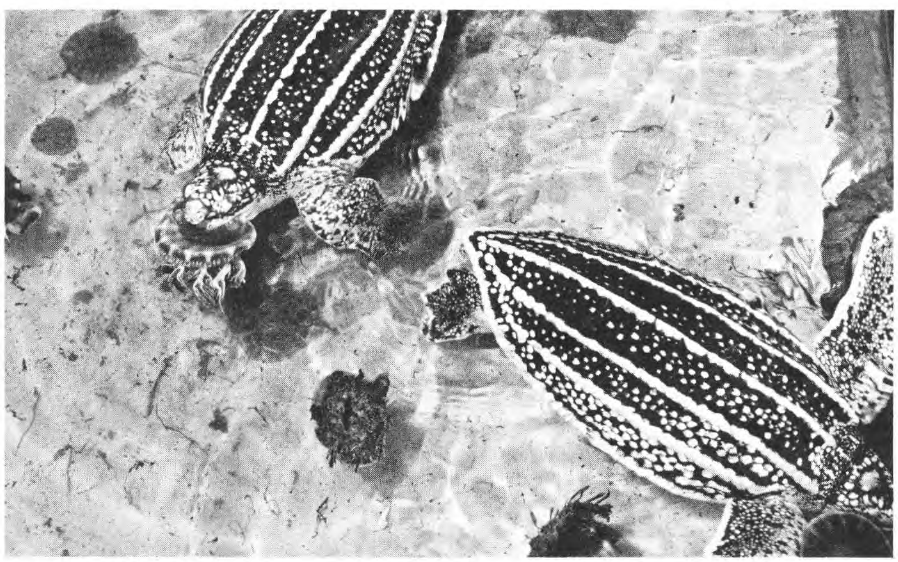
27. We are grateful to Dr. G. E. Cosgrove for all pathology on these specimens.

MIAMI SEAQUARIUM'S LEATHERBACK TURTLE

Pat Foster

Miami Seaquarium is presently the home of a leatherback turtle, Dermochelys coriacea, hatched June 26, 1973. This turtle is extremely difficult to maintain in captivity as previous records testify. In fact, the only other moderately successful attempt to raise this turtle was in 1935 in Ceylon by biologist Deraneyagala. After 662 days, he succeeded in raising one specimen to a weight of approximately 16 pounds. Our turtle, after 17 months, weighs 51 pounds.

At Seaquarium, we have attempted to raise this species of turtle since 1967. Using a small number of turtles loaned to us each year by the Florida Department of Natural Resources, we have tested different diets and tank arrangements with relatively little success. In 1973, however, we began feeding our new batch of seven leatherback hatchlings solely on a single species of jellyfish, Cassiopea xamachana.



Two leatherback turtles at six months of age injesting the jellyfish, Cassiopea xamachana. Photo by Wometco Miami Seaquarium.

During the first year of our surviving turtle's life it was maintained in a foam-padded pool with water temperature heated to 24°-28°C. The turtle was given a bi-weekly dip in potassium permanganate and all abrasions were treated topically with gentian violet. It was supplied with an ad libitum diet of jellyfish supplemented with a multiple vitamin solution injected into the medusa. More details of this turtle's early maintenance and growth are found in the International Zoo Yearbook, 1975. At present, the turtle is residing in an indoor pool with nylon-net protected sides and constant long-wave ultraviolet irradiation.

This turtle is remarkable for its unadaptability to its environment. After 17 months in a confining tank, it still has not learned to refrain from continuously swimming into the sides. Although we have protected it from injury somewhat with foam padding and nylon netting, the rostrum and front edge of its flippers are incrusted with callouses and sores. It divides its time between feeding on the jellyfish always present in its pool and swimming against the sides. On occasion, it is seen resting at the surface, but less often than loggerhead or green turtles. Twice this turtle has been observed floating on its back for periods of about two minutes. When startled, it will sometimes roll onto its back, but it quickly rights itself. This leatherback turtle, unlike all other species of sea turtle in captivity, does not orient towards the person who feeds it, not even at feeding time. It just continues to swim against the sides until it is startled by the sound of the jellyfish being added to the water, whereupon it begins to eat.

Some of the International Union for the Conservation of Nature Sea Turtle group who viewed the leatherback turtle at Seaquarium in November, 1974. Left to right: Peter Pritchard, George Hughes, Archie Carr, L.D. Brongersma, George Balazs, and the author holding the turtle.

Watching these turtles in captivity for any length of time causes one to wonder about their life at sea. How do they locate food, or is it merely luck which brings them into contact with enough pelagic jellyfish to sustain them?

Possibly mature leatherback turtles supplement their jellyfish diet with fish or other foods that they cannot digest as hatchlings. The leatherback turtle's distribution is worldwide; it has been reported as far north as Canada, but nests only in the tropics and subtropics. It is endowed with a thermoregulatory system of blood vessels to its limbs that act to conserve body heat. It is found that these turtles maintain a body temperature as much as 18°C above ambient water temperatures. Internal telethermometer readings made on our turtle here at Seaquarium will help to shed light on this phenomena.

Little is known about the behavior of these animals in their own environment. In fact, immature leatherbacks beyond the hatchling stage have never even been observed. Mature leatherback turtles are rarely spotted at sea and only a few of their nesting beaches have been studied. We are hoping our work with this captive leatherback turtle will illuminate some factors about their biology and behavior that is so far unknown.

The author thanks Ross Witham and the Florida Department of Natural Resources for working with Miami Seaquarium on this leatherback turtle project. Without their assistance this project would not have been possible.

BIBLIOGRAPHY

Deraneyagala, P.F.P. (1939) The Tetrapod Reptiles of Ceylon, Ceylon Journal of Science, 1:1-412.

Foster, P.F. and C. Chapman. (1975) International Zoo Yearbook, Zoological Society of London.

Friar, W., R.G. Ackman and N. Mrosovsky, (1972) Science. Vol. 177, pp. 791-793.

Witham, R.E. (1973) Journal of the American Veterinary Medical Association. 163(6):656.

INVESTIGATION OF SODIUM CHLORITE FOR USE IN MARINE SYSTEMS

Bruce M. Poole
Curatorial Department, New England Aquarium

Introduction

Sodium chlorite was first used in industrial applications some years ago. It was found useful in bleaching processes of everything from cherries to paper pulp. When Olin Chemicals took over large scale production of sodium chlorite, other applications were tried and tested. Paper mills found that sodium chlorite ($NaClO_2$) not only bleached pulp well, but also kept bacterial slime from fouling their lines. Industries with cooling towers used $NaClO_2$ as an algaecide, saving expensive cleaning or use of chelated copper sulfates. Studies done by Olin established a very low toxicity level of $NaClO_2$ to fish, making it even more desirable for use in industries that have waterway discharges.

With proper application $NaClO_2$ can have many uses in Marine systems. Chemically it is very stable in systems with a pH above 7. Once the initial organic combinations are over, levels are stable for days to weeks depending upon application. Toxicity is only a problem at a pH below 6, where chloramines and hydrolysis products can develop, and in the presence of ozone or direct sunlight where chlorate may be formed. Marine fish have been kept in duration baths of 60ppm with no ill effects.

The first uses of $NaClO_2$ in marine systems are documented by Dempster (1971). He states that a 5-25 ppm level of $NaClO_2$ over a period of seven to ten days will successfully cure salt water ick and cryptocaryon; as well as being effective against flukes at 15-25ppm. However, $NaClO_2$ has one drawback, it is bactericidal. In effecting a successful cure for cryptocaryon or flukes, it may destroy the filter bacteria, causing waste accumulation and build up of toxic NH_3. This bactericidal nature of $NaClO_2$ can be of value in another type of marine system. Marine mammal systems, because of problems with bacteria and fecal wastes, have been using hypochlorite (HOCl) at low levels of .2-.4ppm for disinfection. Hypochlorite is an undesirable compound with which to work. In water equilibrium it exists as a gas and hypochlorous acid, which means it smells like a swimming pool, acidifies the water and is constantly escaping. Most applications use HOCl on a constant feed basis, or at least 4-6 times a day, making it expensive and time consuming. HOCl also is an eye and nose irritant and has the ugly property of combining with hydrocarbons to form chlorinated hydrocarbons. These chlorinated compounds are often insoluble in water and form an obnoxius brown surface scum. On the other hand, $NaClO_2$ is also bactericidal, but stable, non-toxic and oxidizes most hydrocarbons with its possible valence change of 7. (Garibaldi, 1970).

To investigate these possible uses of $NaClO_2$ three projects were done at New England Aquarium. The first involved the in vitro testing of sodium chlorite's effect on NH_3 utilization by filter bacteria. Another test was an actual application of $NaClO_2$ for treatment of flukes with constant monitoring of tank NH_3. The third project was direct sensitivities of certain bacteria to $NaClO_2$, to substantiate its bactericidal nature.

Part I: <u>Sodium Chlorite's Effect on Ammonia Utilization by Filter Bacterium</u>

Sodium chlorite's therapeutic use against fish parasites is of little value if the biological equilibrium of the tank is seriously altered. Ammonia is a fish waste, normally converted to nitrite mainly by <u>Nitrosomonas sp.</u>, a filter bacteria. Ammonia (as NH_3 at varying percentages with varying pH) causes repiratory problems at 300ppb and death at 1,000ppb (Burrows 1964). Since $NaClO_2$ is bactericidal, the destruction of nitrifying bacteria and resultant rising NH_3 may defeat the treatment process.

To test this, four identical tanks were set up and inoculated with filter bacteria. NH_4Cl was added daily to "feed" the Nitrifiers. When populations were developed that could utilize a certain amount of ammonia in twenty-four hours, levels of chlorite were established in three of the tanks. The fourth tank remained as a control. All tanks had ammonia and chlorite measured every twenty-four hours with additions of "food" (NH_4Cl) and necessary chlorite made after measurement. The effect of chlorite on NH_3 utilization was measured as remaining NH_3 after twenty-four hours. This was compared to values for NH_3 remaining before the addition of chlorite. All ammonia readings were done as total $N(NH_4^+$ and $NH_3)$ with an Orion 10-95 ammonia gas sensing electrode and specific ion meter. Chlorite analysis was a modification of the Black and Whittle determination for free and available chlorine (Dempster 1970).

Bioassay Specifics:

Metaframe 10 gallon all glass tanks
Metaframe undergravel filters #160D
surface area 0.725ft. (1165cm)
Airlifts 2/tank, 1/4" diameter, 5.5" long
average flow rate 0.75 gal/min (2800ml/min.)
#2 New Jersey sand (size 3-5mm) 2"/tank
251 salt water
(Water Quality during Bioassay)
salinity 30-32ppt
pH 7.6 - 7.7
temp. 20-22°C
Seed inoculum of 500g "old" sand (from active system)

For ten days all four tanks were fed 10ppm NH_4Cl per day. Readings each day, before feeding, showed consistent levels around .100ppm. Thus the bacteria in the filter were using 10ppm - interface loss per day. Interface loss was tested using the same tanks with sterile sand. The loss is greatly effected by atmospheric conditions, but for the purpose of discussion an average loss of 7.7ppm/day was computed. After the reading on day ten, chlorite levels of 5, 10, and 25ppm were established in three tanks. NH_4Cl additions were continued in all four tanks for four more days. The following chart (Figure 1) presents the data obtained:

Figure 1

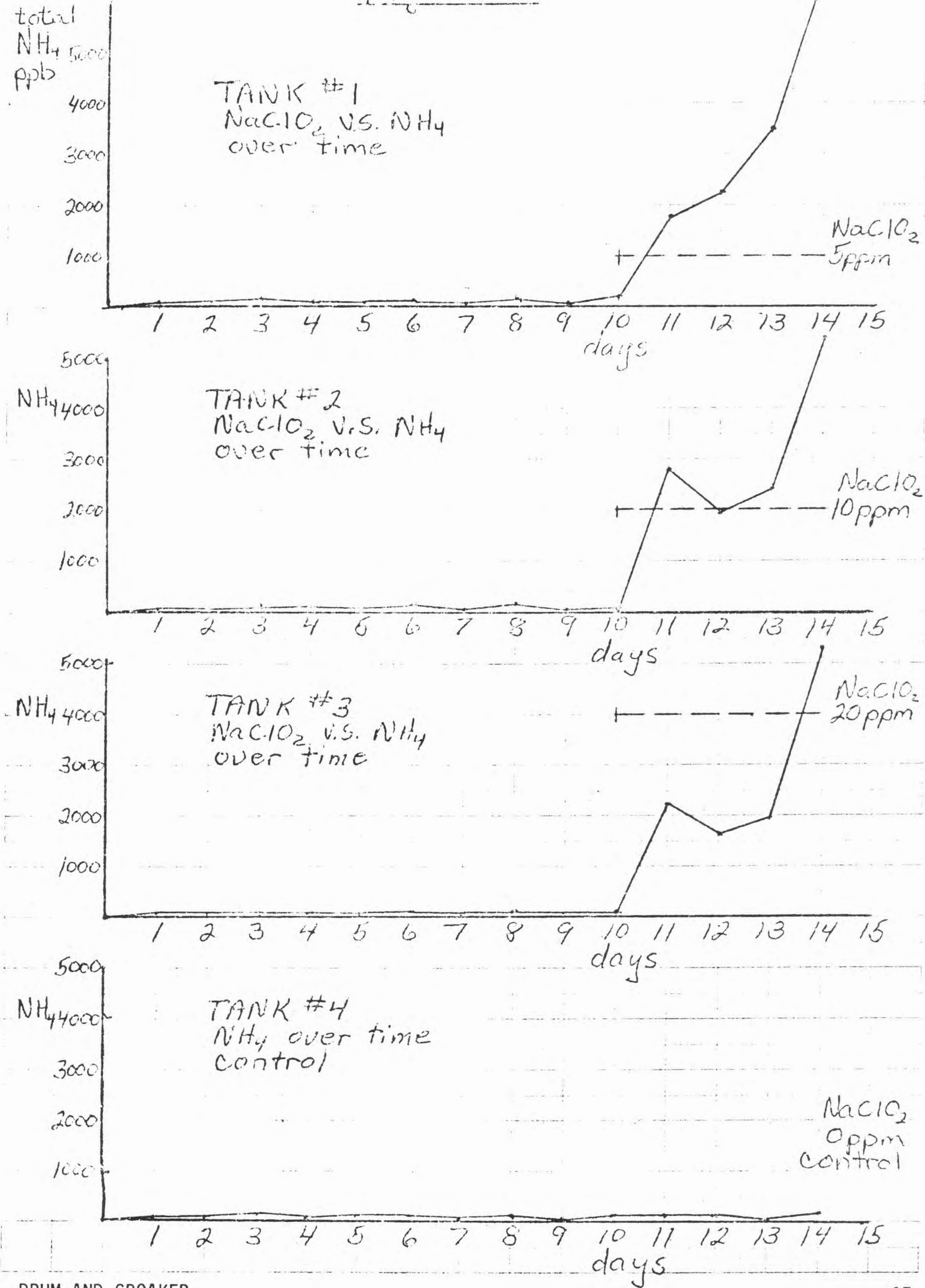
DAY#	TANK #1		TANK #2		TANK #3		TANK #4	
	NH_3 (TOTAL N)	ClO_2	NH_3	ClO_2	NH_3	ClO_2	NH_3	ClO_2
1	84ppb	0	78ppb	0	78ppb	0	56ppb	0
2	134	0	78	0	80	0	62	0
3	168	0	182	0	180	0	126	0
4	108	0	119	0	125	0	92	0
5	99	0	87	0	82	0	60	0
6	127	0	166	0	148	0	103	0
7	61	0	82	0	88	0	76	0
8	184	0	160	0	155	0	140	0
9	40	0	76	0	64	0	45	0
10	196	5ppm	56	10ppm	93	20ppm	129	0
11	1,792	5	2,800	10	2,240	20	140	0
12	2,240	5	1,960	10	1,680	20	126	0
13	3,500	5	2,400	10	1,945	20	85	0
14	6,720	5	5,040	10	5,320	20	205	0

10ppm NH_4Cl was added to each tank every day after the reading was taken. Chlorite was brought to its respective levels every day if necessary. Graphic representation of the NH_3 levels is found in Figure 2.

Conclusion

Sodium chlorite in levels of 5, 10 or 20ppm markedly effects NH_3 utilization by filter bacteria. This is noted by significant rises in NH_3 levels of tanks with ClO_2 levels. The question still remains as to whether the bacteria were actually killed. After draining the tanks and

Figure 2



total NH4 ppb
5000
4000
3000
2000
1000
TANK #1
NaClO2 V.S. NH4
over time
NaClO2
5ppm
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
days
5000
NH4 4000
3000
2000
1000
TANK #2
NaClO2 V.S. NH4
over time
NaClO2
10ppm
1 2 3 4 6 6 7 8 9 10 11 12 13 14 15
days
5000
NH4 4000
3000
2000
1000
TANK #3
NaClO2 V.S. NH4
over time
NaClO2
20ppm
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
days
5000
NH4 4000
3000
2000
1000
TANK #4
NH4 over time
control
NaClO2
0ppm
control
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
days

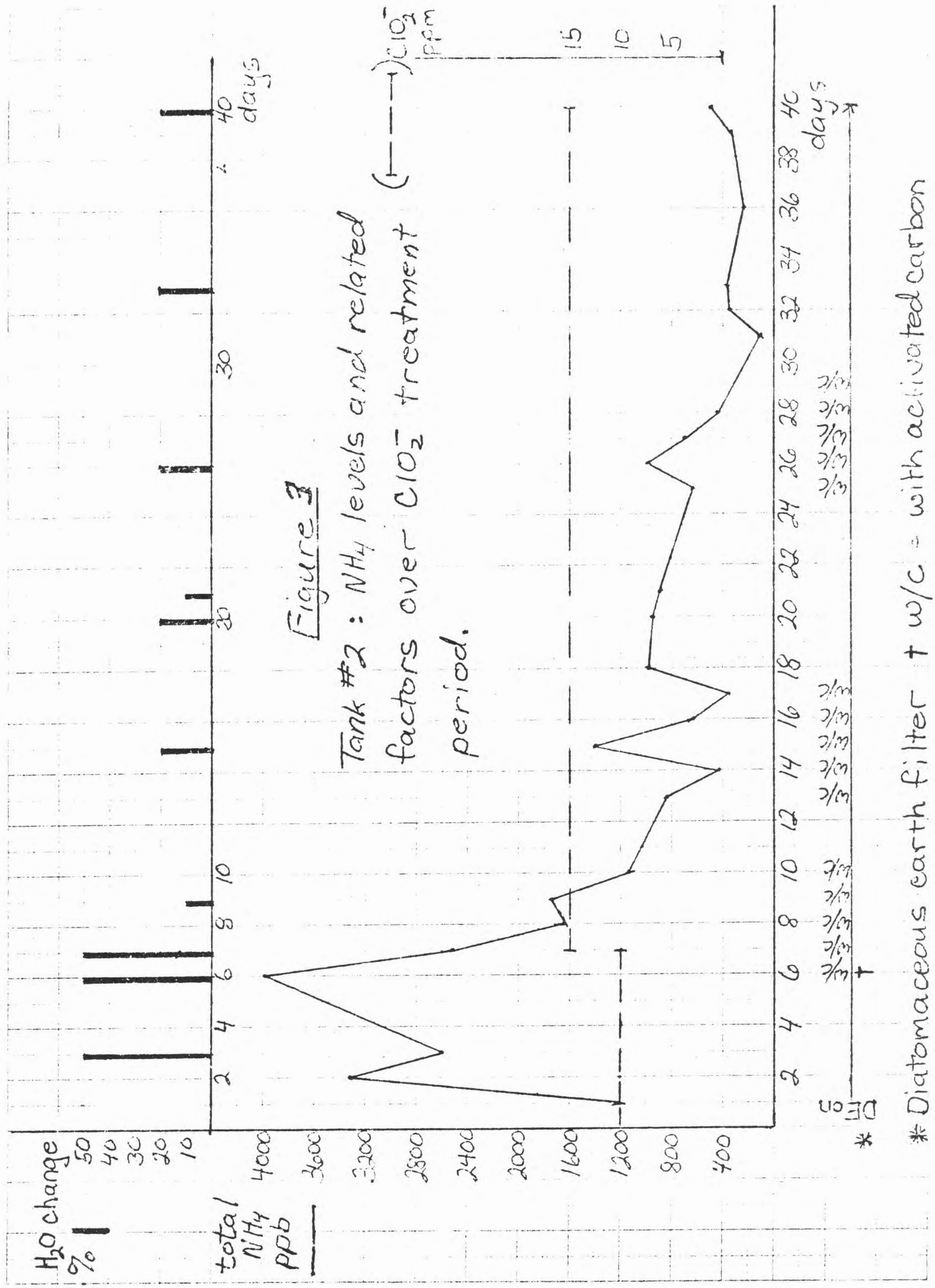


Figure 3

Tank #2 : NH_4 levels and related factors over ClO_2^- treatment period.

refilling them with new salt water, a period of three days monitoring showed no return to NH_3 utilization. It is assumed, therefore, that the bacteria were destroyed. Further tests involving direct sensitivities of pure cultures to sodium chlorite are being undertaken to substantiate this.

Part II: Tank Observations Over Prolonged Sodium Chlorite Treatment

This experiment was designed to give us information on chlorite in two areas: to establish its treatment efficiency against Benedinium melitus (flukes) and provide data on chemical changes in a tank system over prolonged treatment. To control adverse effects, water quality was closely monitored, and corrected with water changes and Diatomaceous earth filtration. Benedinium m. had been observed months before in this display tank. Repeated applications of Dylox (1ppm) kept the parasite at bay, but eggs in the filter gravel continually reinfested the tank. Benedinium had been observed for six days on the fish eyes when it was decided to do a prolonged treatment with $NaClO_2$.

The semi-circular display tank had one 3'x5' glass window and contained 560 gallons. It had been set up for some time as a Temperate tank with Chondrus crispus and a variety of Spadefish (8), Jacks (3), Sea catfish (5), and Snook (1). The biomass was 7.05 kg. (Throughout treatment they consumed .020-.150 kg. food/day.) An undergravel filter of oyster chip and #2 sand covered the bottom of the whole tank. (28 sq. ft.; airlifts (2); flow rate=19 gal/min.) The plan was to establish slowly a $NaClO_2$ level of 15ppm, monitoring chlorite, NH_3 and tank conditions very closely. Chlorite was maintained at levels desired by addition as needed. Chlorite analysis was a modification of the Black and Whittle determination for free and available chlorine (Dempster 1970). All ammonia readings were done as total $NH_3(NH_4^{+}+NH_3)$ with an Orion 10-95 ammonia gas-sensing electrode and specific ion meter. Figure 3 is a graphic representation of chlorite and ammonia levels with comments throughout treatment.

In this treatment application of ClO_2 it was interesting to note several system reactions. The ammonia level decreased through the course of treatment. After one day the ammonia rose to 3304ppb and peaked on day six at 3990ppb. With the system at a pH of 7.6 the percentage unionized (toxic) NH_3 was 1.56% (or 62ppb at 3990ppb). This is below the stated level of 100ppb for respiratory problems (Burrows 1964). During the first seven days three 50% water changes were done, but after that 20%/week kept the ammonia level decreasing. From day fifteen to the final day (forty) the ammonia never exceeded 1000ppb (15.6ppb unionized). The lower than expected ammonia levels are attributed to the constant use of a large Diatomaceous earth (DE) filter. Filter intake water was drawn from under the filter bed and filter excurrent released into the front of the tank. The DE filter was recharged periodically with powdered activated carbon for further absorption and clarification. Assuming the nitrifying bacteria had died (Part I) the DE filter was able to remove fish wastes in particulate and colloidal forms, preventing toxic ammonia build up. Because of the necessary frequency of DE cleaning in the first eight days, it is assumed that the initial ammonia rise is due to the breakdown and release of the organic filter accumulations. Salinity, pH and temperature were stable during treatment, remaining at 32ppt, $7.6 \pm .1$ and $68^{o}F$ respectively. The Chondrus crispus in the tank had completely broken down by day twenty-five, necessitating siphoning and DE filtration with carbon to remove the brown color (phenols). A problem in the experimental was the development of a bacterial infection characterized by white slime accumulations and split fins (suspected Aeromonas; Psuedomonas). First observed around day twenty, the infection was held in check with meal gelatin (medicated food) Terramycin (75mg/g). The overall treatment was successful; after six days all visible Benedinium melitus were gone and no reoccurrence has been noted for three months since termination of the treatment.

Part III: Direct Sensitivities of Certain Bacteria to Sodium Chlorite

Possible applications of sodium chlorite extend even into Marine Mammal systems, where there is a constant problem of fecal waste and bacteria build-up. Since such build-up can cause sores and infections, disinfection of the system is usually required. Chlorine is commonly used, but is not a desirable compound. As mentioned in the introduction, it is very unstable, and disrupts natural water chemistry. Since chlorite, in many ways is superior to chlorine, the purpose of Part III is to substantiate chlorite's bactericidal properties and prove it is as effective as chlorine.

Sensitivities were done similar to phenol coefficient studies, where exposure vs. death of organism is derived (Bartholomew 1971). The test involves organism exposure to known concentrations of chlorite in distilled water over set periods of time. A loopful of organisms are removed after a set time from test concentrations and inoculated into nutrient broth. If growth occurs, then chlorite was not bactericidal over that period of time. If no growth occurs, chlorite was bactericidal. Controls were run in distilled water. Normal tests on such disinfectants run over time periods of minutes, but chlorite is not quick acting in low concentrations. In system applications of low concentrations of chlorite, organisms are constantly exposed to the chemical. For this reason the time periods to be tested were set at one, four, twelve and twenty-four hours.

Results showed Staphylococcus aureus and Escherichia coli sensitive to chlorite, while spores of Bacillus cereus were not sensitive. (Figure 4) Of the concentrations tested (5, 10, 25 and 50ppm ClO_2^-) none were shown to be rapid in their disinfection properties. But with constant levels present it is not necessary to be rapid; most bactericidal agents are bacteriostatic at lower levels or over shorter than lethal time. (Franklin and Snow, 1971) This means that with chlorite in the water, bacteria are prevented from multiplying due to complexing of ClO_2^- with the cell membrane.

Therefore, sodium chlorite is bactericidal and does perform the same functions as chlorine. It also has properties more condusive to maintaining healthy marine mammal systems. The next test, of course, is to try it.

Figure 4

	ClO_2	Exposure Time 1 hour	4 hours	12 hours	24 hours
E. coli	5ppm	+	+	-	-
	10	+	+	-	-
	25	+	-	-	-
	50	+	+	-	-
	control 1	+	+	+	+
	control 2	+	+	+	+
S. aureus	5ppm	+	-	-	-
	10	+	-	-	-
	25	+	-	-	-
	50	+	-	-	-
	control 1	+	+	+	+
	control 2	+	+	+	+
B. cereus	5ppm	+	+	+	+
	10	+	+	+	+
	25	+	+	+	+
	50	+	+	+	+
	control 1	+	+	+	+
	control 2	+	+	+	+

\+ growth
\- no growth

Literature cited

Bartholomew, J. R., Laboratory Textbook and Exercises in Microbiology, p. 116-120, Wm. Brown Co., Dubuque, Iowa, 1971.

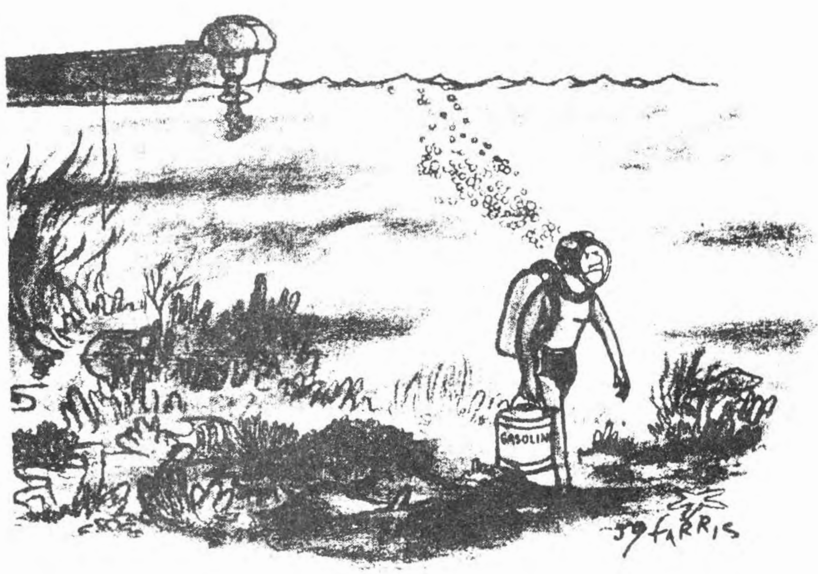
Burrows, R. E., "Effects of Accumulated Excretory Products on Hatchery-Reared Salmonids," Bureau of Sport Fisheries and Wildlife, Research Report #66, 1964.

Dempster, Robert P., "Modern Methods of Cure," Anchor, Volume 5, No. 2 pp. 59-61, 1971.

Dempster, Robert P. and Anderson, Bryce, "Description of the Methods Used to Determine the Presence of Chlorite...in Fish Tanks," Anchor, Vol. 4, No. 10, pp. 338-341, 1970.

Franklin, T. J. and Snow, Biochemistry of Antimicrobial Action, pp. 21-29, pp. 47, Academic Press, 1971.

Garibaldi, Louis E., "Chlorite +3," Anchor, Vol. 4, No. 10, pp. 335-337, October 1970.



AN OVERVIEW OF MYSTIC MARINELIFE AQUARIUM

The Mystic Marinelife Aquarium, with 2,000 water creatures inhabiting its unique new exhibits, opened to the public in the fall of 1973.

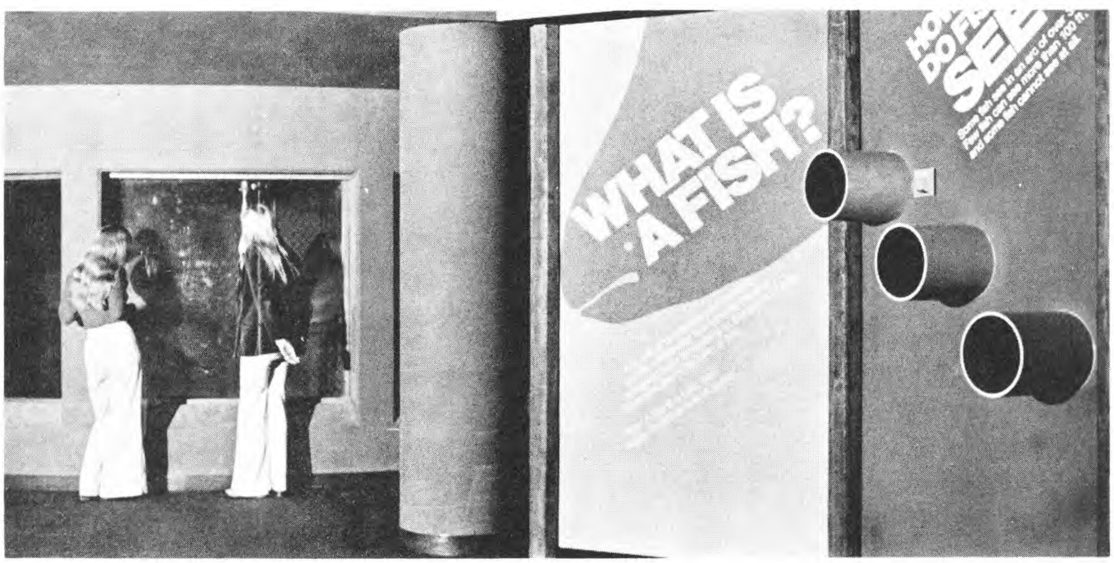
Visitors to the new $6 million educational and recreational institution will find a unique way of viewing the water world and its creatures, a method of exhibiting aquatic animals that has taken years to perfect. Not only do the exhibits at the Mystic Marinelife Aquarium bring visitors face to face with water creatures, but they display these animals in ways that enhance understanding of how they--and ultimately, all life--relate to the environment that permits them to exist.

The exhibits of live animals--ranging from menacing sharks to shiny little silversides--are coupled with a sophisticated array of audio-visual presentations, graphics that educate and entertain, and works of art to create a complete picture of the ecology of the water world, particularly the sea.

Mystic Aquarium is operated by Aquarium Systems, Inc., of Eastlake, Ohio--one of the most respected companies in the field of aquarium management. Aquarium Systems also operates the Aquarium of Niagara Falls, USA.

Located on a twelve acre landscaped site, Mystic Aquarium is housed in a two-level building of buff-gray, pre-cast concrete panels, that overlooks a one acre lake. In spring and summer koi carp and native water fowl can be seen in the lake.

Mystic Aquarium is open year-round and encourages the utilization of the Aquarium by educational groups.

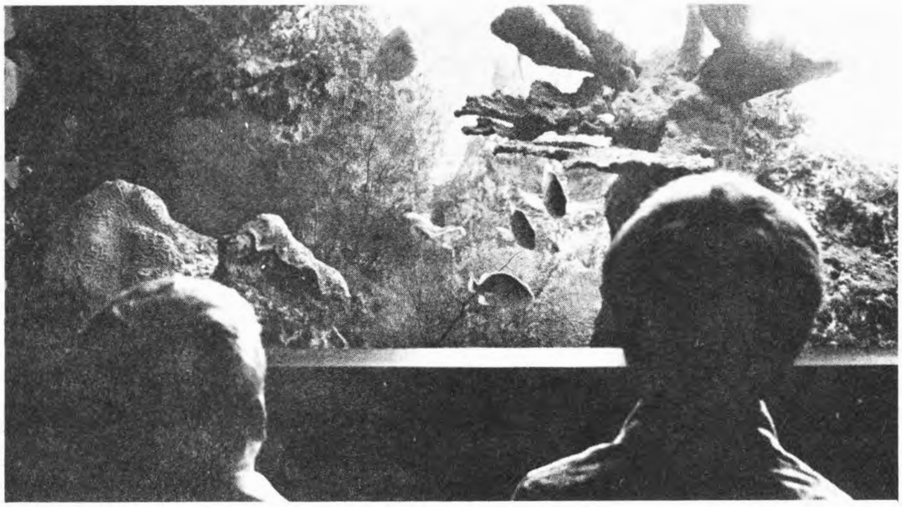


Visitors to the Mystic Marinelife Aquarium are given a fish's-eye view of the world as they peep through the viewing ports at this educational exhibit. Special lenses in the ports simulate the eye of three different types of fish. The viewer sees the world as the fish would.

Six dolphins are on hand at the Aquarium, participating in behavioral demonstrations before an audience of up to 1400 people. The dolphins perform in a 400,000 gallon saltwater Marine Theater. When not performing, the dolphins greet visitors who approach them by tail-walking in the water and splashing the surface with their broad flukes.

An exhibits crew prepares to install a reef exhibit. Experts in aquarium design have simulated a wide variety of aquatic habitats in the Aquarium's exhibits.

The Aquarium is divided into three main exhibit areas, each arranged according to evolutionary and ecological concepts. The first shows how, over millions of years, animals have evolved special characteristics that enable them to survive in the water. Another section shows the different underwater communities present in North American waters.

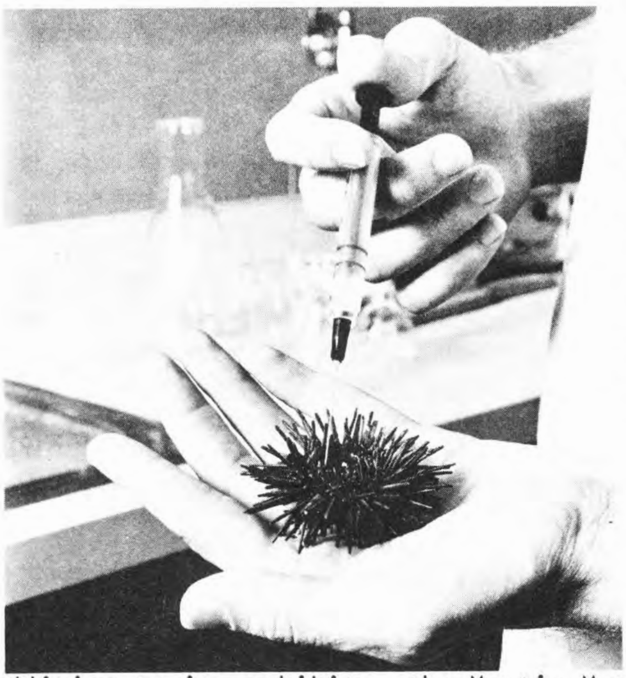


Two youngsters marvel at the multi-colored life of the coral community. Two coral reef exhibits, one of the reef by day, the other the reef at night, show visitors to the Aquarium the differences in reef life during the hours of light and darkness.

With a few exceptions, most of the exhibits house creatures native to the New World, from the warm waters of tropical seas to the chill oceans of the Arctic. Within this vast portion of the Earth's surface live a fantastic variety of water animals: Common animals such as the bluegill sunfish, beautiful creatures such as the French angelfish, and weird denizens of sea caves such as the giant Pacific octopus.

One of the most exciting exhibits is a 30,000 gallon display situated in the middle of the Aquarium's first level. It houses creatures of the open sea. Here bluefish flash through the water while huge sharks and graceful sting rays prowl as they do in the open ocean.

Mystic Aquarium uses a synthetic sea water developed by Aquarium Systems, Incorporated for its marine exhibits. The preparation, "Instant Ocean", is manufactured and sold by Aquarium Systems and is used on a world-wide basis in laboratories, industry, public aquariums and home aquariums where highly controlled environmental conditions are required.



The sea urchin is an important creature to scientists at Mystic Aquarium, for it enables them to test the quality of water in the Aquarium's exhibits. Here, potassium choloride is injected into an urchin to make it release its eggs. Fertilized eggs are placed in test samples of water. The fertilized urchin egg is very sensitive to water conditions. Proper development of the eggs indicates that water quality is satisfactory.

In addition to its exhibits, the Mystic Marinelife Aquarium has an education department, which works with schools throughout the state, an exhibits department and a laboratory of marine biology that conducts research and at the same time conducts quality control tests that assure the health of animals exhibited at the Aquarium.

DRUM AND CROAKER

THOUGHTS ON PLANNING A ZOO AQUARIUM*

U. Erich Friese, Curator of Fishes
Taronga Zoo

and

Ronald Strahan, Director
Taronga Zoo

INTRODUCTION

The only major aquarium in Australasia, in Taronga Zoo, has reached the end of its structural life and must be replaced. Our thoughts on the principles that should guide the design of the new aquarium are stimulated by this necessity but may have a rather wider application. Since we believe that the great cities of the Australasian region should all have good public aquaria - ideally as departments of their zoos - we feel that our approach to the planning of a large aquarium may be of more than local interest.

THE CONCEPT

Although a few zoos include major public aquaria of recognised excellence, the traditional zoo aquarium is often an insignificant building with about two dozen tanks, situated somewhere between the giraffe house and the seal pool: in one well known zoo the lion house served as a "temporary" aquarium for many years. Although many zoological gardens are outgrowing their early role as mere animal repositories, the zoo aquarium usually still consists of an unplanned collection of exhibits with little relevance to the modern zoo's functions in education, research and wildlife conservation.

As evidenced by such zoos as London, Frankfurt, Berlin, New York and Tokyo, a large aquarium can be a popular attraction. With an ecological conscience growing rapidly among the public - fostered by underwater film and TV adventures and news reports of environmental pollution - there is an increasing public awareness of the aquatic environment.

A public, which is seeking insight into basic biological concepts, is not well served by traditional zoos or aquaria. It is not enough

---

*REPRINTED from "Bulletin of Zoo Management", Vol. 3. No. 2, June 1971

simply to display animals in rows of rectangular, barred cages with little signs giving the countries of origin and the Latin names of the species, nor is it sufficient to offer the aquarium visitor an arbitrary exhibit of fish species A, B and C in one tank and X, Y and Z in the next. The aquarium curator does not dispose of his responsibilities by putting the label, Paracheirodon innesi, on a tank of Neon Tetras: visitors would like to know why this fish is so brilliantly coloured or, more generally, what produces the colouration - an enquiry which can easily lead into a general and far-reaching explanation of the function of chromatophores and the processes of Natural Selection.

In setting up any exhibit in an aquarium, the aquarist should ask himself, "What can a visitor learn from this?" This is not to say that beautiful or uncommon aquatic creatures should be excluded from display; but that these qualities are not sufficient in themselves to merit the exhibition of certain creatures.

There is virtually no limit to the range of biological topics that can be demonstrated or commented upon in relation to any exhibit - a point well made by Conway (1968) in his provocative essay on the display of a Bullfrog. However, a properly planned aquarium might well begin by providing exhibits that illuminate the implications of pollution and destruction of the environment, the structure of animal communities, animal societies, aggression, and the ways in which species are adapted to their environments, and cope - or fail to cope - with natural or man-made changes in their habitats. It would also be pertinent to provide insight into the principles involved in the wise utilizations and management of populations of commercially exploited aquatic species. The concept of the maximum sustainable yield of a mullet, tuna or lobster fishery should be made clear to the visitor before he passes through the exit of a zoo aquarium.

By following this line of approach to the display of aquatic specimens one can also make a good case for going beyond the normal macroscopic level of display and observation. An aquarium is concerned with water, and a visitor could well be introduced to the concept of the hydrological cycle; from its atmospheric origin, via the soil to rivers, into the oceans, and back again to the atmosphere. For such an exhibit, reduced to microscopic levels, it would be appropriate to utilize optical and television microscopes and projection equipment to extend the view of the visitor.

These are not absolute prescriptions. No two aquaria can, or should, be identical either in layout or philosophy, but no aquarium of the future can be regarded as fulfilling a purpose unless it aims at thematic biological displays. Zoos have lagged behind museums in formulating a reason for their existence. Zoo aquaria have similarly lagged behind their parent zoos for lack of an acceptably defined concept. It has been said, that technological progress over the past decade has brought about a revolution in aquarium "hardware", but it must be admitted that the "software" of the profession is still in its infancy.

THE FINANCIAL BACKGROUND

The economic success of commercial fish menageries, porpoise pools and oceanaria - many of them of low aesthetic and biological standards - demonstrates the existence of a growing public market for aquatic biological displays. The few well-funded and professionally operated aquaria, which combine light-hearted entertainment with serious education, are thriving enterprises despite their relatively high admission fees.

It may be that first-class aquatic exhibits cannot be provided from a normal zoo budget. A separate entrance fee may be necessary either to cover, or to contribute to, the operating costs.

It is easy to fall into the error of regarding the problems of maintenance of aquatic exhibits as essentially the same as those involved in other areas of a zoo but there is a very real difference. Whereas the air surrounding a terrestrial tetrapod is renewed without human intervention, the water surrounding an aquatic animal must be monitored, controlled and renewed. The hour-to-hour survival of most individual animals in an aquarium depends upon the efficiency of mechanical and chemical life-support systems. Like a human being, an aquarium is at the mercy of its "heart", "kidneys" and "lungs": a circulatory occlusion, a failure to remove excretory nitrogen, or a cut-off of the air supply is rapidly lethal.

An aquarium is an elaborate, and therefore expensive, homeostatic mechanism: a "biostat". One can have big biostats or little ones and, as a first principle, this concept of scale must be accepted. If $100,000 is available it is a pathetic mistake to use such funds to build a shell which requires a million-dollar support system: the inevitable compromise can totally destroy the overall concept. We shall advert to this problem later but, as a working guide, it is suggested that no more than two-thirds of the funds available (or confidently foreseen) for the construction of an aquarium be committed, in the first instance, to its construction. The remaining third is likely to be called upon for contingencies.

All this apart, there remains the question: where do capital funds come from? From our acquaintance with reputable overseas aquaria it would seem that, while smaller facilities can be funded from zoo reserves or by special appeals by supporting societies, larger establishments have gained funds from governments and industries by stressing their relevance to research (systematics, physiology, pathology, etc.) or to fisheries. In the Australian context, it would seem reasonable to seek funds from State and Federal conservation, research and fisheries organizations, and from commercial and sporting fishermen, to establish and maintain first-class aquaria which directly or indirectly serve their special interests.

PLANNING AN AQUARIUM

The solidity of an aquarium (large volumes of water are weighty) and its complexity (as a biostat and as a public facility) demand thorough planning prior to presentation of a brief to the contracting builder.

Given an initial concept of function and an order of magnitude of capital expenditure, it must still be recognised that there is no one man in the world to whom a governing body can confidently entrust the total planning of any but a smaller aquarium: the state of the art is such that even those few men who have designed large aquaria cannot be regarded as definitive experts.

Almost inescapable facts of life demand that the design of every new major aquarium be in the hands of a local committee consisting of representatives of local interests and utilizing the services of a local firm of architects. The degree of success of such a committee depends essentially upon its humility since, after it has settled upon the basic purpose of the enterprise and appointed a zoological curator, it must then set out, not merely to pick the brains of the rest of the world, but to learn from the mistakes of every comparable institution. We are conscious that, as zoologists, we have a biased view of these problems but our experience leads us to believe (a) that the zoological head of an aquarium must be appointed before any planning gets underway; and (b) that such a head and the architect should closely examine the functions of comparable institutions before beginning to compile a detailed brief. Such an exercise, involving perhaps thousands of dollars, can save tens or hundreds of thousands of dollars in avoidable errors.

As an example of an approach to planning, it may be instructive to look back on the 1963 progress report of the New England Aquarium Corporation, Boston, which was set up in 1961.

> "....... Progress to Date: The New England Aquarium Corporation has, over an active period of two years, accomplished or set in motion the following activities for the development of the Aquarium:
>
> 1. Developed leadership provided by nine directors and a Board of Trustees of outstanding business, financial and scientific leaders.
>
> 2. Visited over thirty-five aquariums in North America and Europe, consulting with directors, curators and technique consultants.
>
> 3. Studied plans of over forty-two leading aquariums throughout the world. Hired an outstanding aquarium planner and director, together with a nucleus of staff.
>
> 4. Hired an outstanding group of designers, architects and engineers, now at work on the design of the building.

5. Set up a continuing research survey of the aquatic mammals of New England.

6. Made two survey collecting and research trips to the upper Amazon region.

7. Initiated a reciprocal animal trading program with the important aquariums of the country.

8. Established a public aquarium information committee composed of the Curator-Director of this institution and those in New York, San Francisco, Los Angeles and Philadelphia.

9. Prepared and made available brochures and fact sheets. Requested and received newspaper, radio and TV coverage and support.

10. Surveyed trusts, funds, businesses and individuals to gather fund-raising data. These have to date included business and civic leaders, conservationists, sportsmen and leaders in marine and water-oriented industries.

11. Carried out an educational TV program involving eight one-half hour shows on a local commercial station.

12. Initiated and published a technical and informative professional journal, AQUASPHERE, distributed at present in twenty-five states and nineteen foreign countries.

13. Raised operating funds to support the above activities."

This magnificent institution was opened in 1969 after eight years of planning and construction. Although an operation of this scope may be beyond the resources of the average zoo, it is no larger than the more conventional aquaria of the Berlin and Tokyo zoos. Even if one's goal is less ambitious, the same sort of planning approach is called for.

SOME PRINCIPLES OF DESIGN

A public aquarium has to be designed, not only for aquatic animals but also for people.

VISITORS require admission for no less than six hours per day every day of the year, but most come at peak periods of two to three hours' duration, on week-ends and holidays. Thus, for most of the time that it is open, the aquarium is relatively empty and no problems of visitor distribution arise. Nevertheless, design must proceed on the basis of maximum likely visitor density.

The problems of accommodation and circulation of visitors in a closed space are common to all exhibition halls and can only be solved satisfactorily by a one-way route, with provision (if the route is long) of rest areas and side-loops giving access to special, less "popular", exhibits.

While visitors can be forced into a desired circulation by guide rails and the pressure of others behind them, a system that entices them forward is more desirable: a carrot on a stick is better than a whip! This can be accomplished by having exhibits, or groups of exhibits, face the visitor along the desired line of progression.

The traditional "railway carriage" arrangement of tank windows does not encourage such a progression and has the related disadvantage that attention to any one tank is distracted by peripheral vision of exhibits on either side. A "sawtooth" arrangement of tanks, such that each faces the visitor obliquely, permits him to treat each one separately while still being attracted to the next (unseen) one. As is now generally recognised in exhibition design, it is quite unsatisfactory to provide competing attractions on either side of the line of visitor progression. Under these conditions the visitor is like the biblical ass of Buridan, unable to choose between two bales of hay.

The "sawtooth" arrangement of tanks is applicable to a rectangular building, a plane spiral, or a helical ramp, but it is by no means the solution to all display problems. Some conceivable displays (reefs, beaches, inter-tidal zones and other such transitional situations) should be lengthy and should engage the peripheral attention of the visitor.

Because the halls of an aquarium must be dimly lit, they must therefore be enclosed. This leads to problems of ventilation to avoid stuffiness and, in humid weather, to reduce condensation on the glass of cold-water exhibits. Many existing aquaria have experienced considerable trouble from inadequate ventilation during peak periods of summer attendance: this can be overcome by airconditioning.

Allied to the problem of ventilation is that of claustrophobia. Susceptible persons - and their proportion is the population should not be underestimated - are affected by stuffiness, crowding and dim illumination, to the point of extreme discomfort. Their needs can be met to some extent by ventilation, but provision should also be made for them by well-lit lay-bys and, ideally, for ready access to "emergency exits" along the set route. Such refuges should also include toilets, drinking fountains, rest areas and provisions for handicapped people. It need hardly be stressed that, since aquarium halls must be dimly lit, the floor surface and visitor traffic pattern must not involve steps or other impediments.

The necessarily enclosed nature of an aquarium hall and the fact that most of the wall area is made of glass, leads to considerable reflection of sound and thus to a high level of noise. This can be reduced by sound-absorbent ceilings and carpeted floors and walls: the subjective blanketing effect of generating a low level of "white noise" is an experiment worthy of trial.

DRUM AND CROAKER

AQUARIUM STAFF constitute a group of humans who have been notably neglected in many of the older aquaria. The aquarium of Taronga Zoo (the first section of which was opened in 1922) provides a notably bad example of this design fault. Tanks in the older section can only be serviced from the front and (at that) only slim, agile keepers can wriggle into the tanks to clean them or rearrange the "aquascape". Galleries behind the newer exhibits allow a keeper to stand, but his access to the tanks involved some contortionist qualities.

As with other under-designed aquaria in the world, these problems have arisen directly from asking architects to design an aquarium in the absence of direction from, or close collaboration with, the curator who is going to maintain the facility. (Such errors have been committed in many other well-known institutions).

At a more positive level, it must be recognised that the degree of excellence on the public's side of the window depends upon what can be done on the other side, behind the scenes. An adequate service area provides, not only ready access to the display tanks but large "off-exhibit" holding and quarantine areas (ideally providing one-third of the total water volume).

The movement of a keeper from the service area to his tanks should be as unimpeded and natural as that of a turner from the factory floor to his lathe. Since, like his land-based zookeeper counterpart, he must keep his exhibit clean, he should have "plug-in" access to an aquatic vacuum cleaner. He must have over-riding control of the water supply of each tank including flow-rate, temperature, and filtering: the tank itself should have basic controls that will operate automatically in his absence.

A gravity-feed system has obvious advantages (in terms of emergency supply) over a pumped system. The duplication of all circulation services is necessary in order that either can be shut off for maintenance. A triplication of services (or at least the pumps) provides for the occasional emergency when one member of a paired system fails while its alternative is dismantled for maintenance.

Ideally, the water supply of a marine aquarium would be completely without filtration, since the food supply of the many filter-feeders totally depends upon an unimpeded water flow from the sea. Unfortunately, large public aquaria tend to be located near large population centres, with inevitable pollution problems and a mechanism or process to remove impurities from the water supply is usually required. Even in the few locations where this particular aspect of "civilization" is not yet of primary concern, natural turbidities caused by wave action (storms), fresh water run-off, plankton blooms, etc. need to be removed for aesthetic reasons. Often such turbidity problems - if of moderate proportions - can be solved by installing large settling tanks but even these must often be augmented by a filtration plant. A partial solution to the demands of filter-feeding organisms is achieved by a by-pass system which periodically adds untreated sea water to those display tanks concerned. Large marine mammal displays, especially those with underwater viewing facilities require considerable fine filtration, usually involving diatomaceous earth. In extreme cases of turbidity and/or pollution it is possible to organize an aquarium as a water system, with only periodical renewal or "topping-up". However, this raises added problems of metabolite build-up requiring even more elaborate water-treatment plants.

A large aquarium has a large food requirement. Most fishes and other aquatic animals feed upon living food and it is therefore necessary to hold food in a condition of freshness above that acceptable to humans. To thaw frozen food-fishes in a distant part of the zoo for later consumption in the aquarium can lead to changes in palatability and vitamin content and even bacterial contamination: an aquarium must have its own deep-freeze facilities.

Many other requirements of the staff and facilities of a proper aquarium could be laid on the line, but these are relatively minor. Once it is agreed that the "backstage" activities are of high significance, these requirements will be met. Not to establish this high priority is an invitation to failure.

RESEARCH

There are few major marine aquaria in the world which have not been set up for, or have subsequently contributed to research. The simple reason for this is that such aquaria, particularly those of open-circulation design, provide research opportunities denied to most universities and many fisheries organizations. Only in a large aquarium can one provide the "biostat" necessary for controlled investigation into the biology of the more delicate marine or estuarine organisms and - it need hardly be emphasised - the provision of such teaching and research facilities in the form of "wet-bench" laboratories involves by far the cheapest expenditure per square foot of an aquarium facility.

The provision of such inexpensive research space for aquatic biologists enhances the reputation of an aquarium, gives a background of expertise contributing to the success of its special exhibits, and provides an intellectual environment in which the staff become aware of the problems of the system in which they work.

Although it may seem mercenary to advert to the subject, it should be recognised that most grant-giving bodies give their capital equipment to the grant-receiving institution rather than to the individual receiving the grant: the institution stands to benefit in the long run. It is therefore to the advantage of the governing body of a zoological aquarium to provide (directly or indirectly) enticements such as studentships or research fellowships to persons who can contribute to science, and thus to the reputation of the aquarium.

IMPLEMENTATION

Any aquarium design that is copied will incorporate the faults of the model. Any design that breaks new ground will, almost of necessity, incorporate some new errors.

To the extent that such errors are culpable, these may be attributed to communication breakdowns between the planning committee, the aquatic zoologist, and the architect or - possibly - to all three of these having aimed too high and fallen short. Our experience leads us to believe that most shortfalls arise from an isolation of planners from the actual administrators and the foregoing remarks are designed essentially to warn against this divisive approach.

References

Conway, William G. Curator. XI (4) 1968, p.310-318
Taft, Edward A. Aquasphere. I (4) 1963, p. 9

"Gesundtheit!"

NOTES ON SOME PARASITES OF BAHAMA FISHES

Harvey D. Blankespoor, Assistant Professor of Zoology
The University of Michigan-Ann Arbor

To fish enthusiasts and even to those involved in studies of fish parasites, the mere recognition of the amount of parasite infestation and infection comes as a surprise. In May of 1972 some 53 fish, representing 36 species, were collected in the region of North Bimini in the Bahamas and examined for their parasites. Methods for examination were as follows:

Each fish was necropsied using standard dissecting techniques. Host organs were individually isolated in petri dishes containing cold-blooded ringers. Recovered flatworms (flukes and tapeworms) and acanthocephalans (spiny-headed worms) were flattened, fixed with A.F.A. and then stained with Mayer's paracarmine and fast green. Parasitic roundworms and arthropods were fixed with hot glycerine 70% ethanol, cleared in glycerine and mounted in glycerine jelly using a double-coverslip technique.

Final tabulations (Table 1) of this survey indicate that most of the major groups of parasites (trematodes, cestodes, nematodes, acanthocephalans and arthropods) were recovered from these fish. Trematodes were the most abundant of all the groups, occurring in more than 80% of the fish examined; the cestodes were next highest with 47%; nematodes were found in 23%, arthropods in 21% and acanthocephalans in 5.7% (Figure 1).

When this parasite fauna, collected from fish in nature, is compared with that of fish collected in the same regions but held in captivity at the John G. Shedd Aquarium for several weeks, some interesting differences are evident. Most striking is that among the fish that were maintained in captivity even for a short time, many species of parasites occurred at a much lower infection rate. Furthermore, the monogenetic trematodes, which are ecto-parasitic, are usually more frequent on fish in aquaria than one would find on the same fish when taken from the ocean. These trematodes may be more numerous on the captive fish because they have a direct life cycle and can easily reproduce in aquaria. The digenetic trematodes (which are usually endoparasitic) often require one or more intermediate hosts (commonly mollusks) to complete their life cycles. These more complex relations are not likely to occur in the exhibit aquaria.

Figure 1. The number (in percentage) of major groups of parasites recovered from 53 Bahama fishes

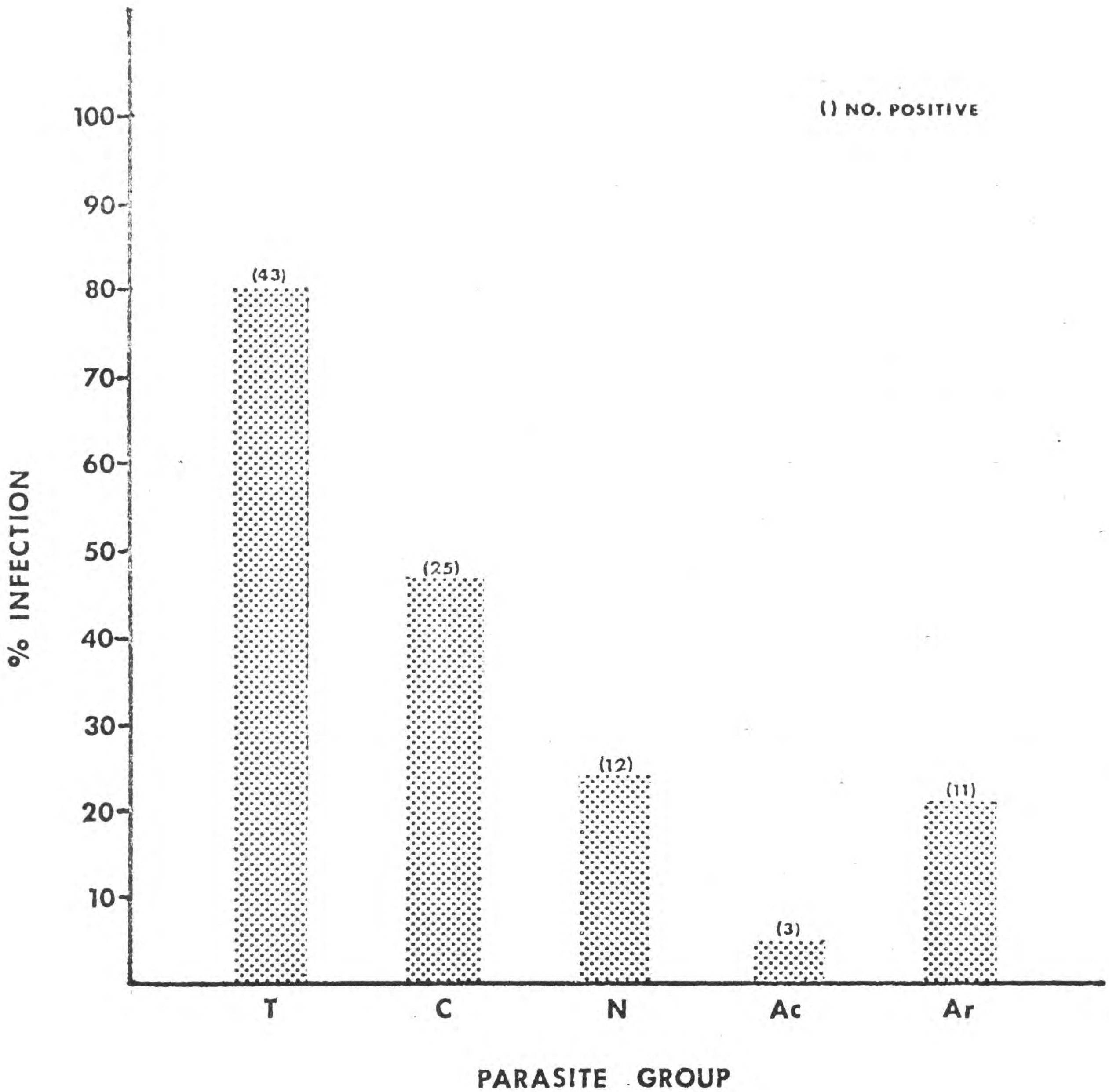


Table 1. Summary of Metazoan parasite groups recovered from 53 Bahamian fishes.

Fish Species	# Exam.	T	C	N	Ac	Ar
Arius felis (Linnaeus) (Sea catfish)	1	a	1			
Selar crumenophthalmus (Bloch) (Bigeye scad)	3	a_3				a_3
Astrapogon stellatus (Cope) (Conchfish)	2	a_2	a			
Seriola dorsalis (Gill) (Yellowtail)	1	a		1		
Caranx latus Agassiz (Horse-eye jack)	1	a				
Epinephelus striatus (Bloch) (Nassau grouper)	2	a_2	al	1		
Scarus guacamaia Cuvier (Rainbow parrotfish)	1	a				
Caranx ruber (Bloch) (Bar jack)	1	a				
Haemulon flavolineatum (Desmarest) (French grunt)	1	a				
Haemulon plumieri (Lacepede) (White grunt)	1	a		a		
Halichoeres bivattatus (Bloch) (Slippery dick)	2	a_2	a_2			
Thalassoma bifasciatum (Bloch) (Bluehead)	1	a	a			
Pomacentrus fuscus Cuvier (Dusky damselfish)	1	a				
Canthidermis sufflamen (Mitchill) (Ocean triggerfish)	1	a	1			
Seriola dumerili (Risso) (Greater amberjack)	2	a_2	al	a		
Diodon hystrix Linnaeus (Porcupinefish)	1	a				
Lactophrys bicaudalis (Linnaeus) (Spotted trunkfish)	1	a				
Pempheris schomburgki Müller and Troschel (Glassy sweeper)	2	a_2				
Calamus bajonado (Bloch and Schneider) (Jolthead porgy)	1	a				
Chaetodon striatus Linnaeus (Banded butterflyfish)	1	a				
Sparisoma aurofrenatum (Valenciennces) (Redband parrotfish)	1	a				
Gramma loreto (Poey) (Fairy basslet)	2	a				
Lutjanus griseus (Linnaeus) (Gray snapper)	1	a				a
Scomberomorus surinamensis CAVALLA (Cuvier) (King mackerel)	1		1	a		
Anisotremus surinamensis (Bloch) (Black margate)	2	a		a	1	

(Table 1 continued)

	# Exam.	T	C	N	Ac	Ar
Coryphaena hippurus Linnaeus (Dolphin)	5	a	l_5	a	a	a_4
Pseudupeneus maculatus (Bloch) (Spotted goatfish)	1	a	l			
Priacanthus arenatus Cuvier (Bigeye)	1	a	a			
Tetrapturus albidus Poey (White marlin)	2	a	a_2		a	a_2
Carapus bermudensis (Jones) (Pearlfish)	1					
Bothus ocellatus (Agassiz) (Eyed flounder)	3	a_3	$a_2 l_2$	$a_3 l$		
Monacanthus ciliatus (Mitchill) (Fringed filefish)	2	$a_2 l$		l		
Thunnus thynnus (Linnaeus) (Bluefin tuna)	1	a	a			
Hemipteronotus splendens (Castelnau) (Green razorfish)	1	a				
Halichoeres poeyi (Steindachner) (Blackear wrasse)	1		a			
Rypticus bistrispinus (Mitchill) (Freckled soapfish)	1	a				
TOTALS	53	43	25	12	3	11

T = Trematode
C = Cestode
N = Nematode
Ac = Acanthocephalan
Ar = Arthropod
a = Adult parasite
l = Larval parasite
Subscript = Number of hosts

An expression of appreciation is due to the John G. Shedd Aquarium crew of the "Coral Reef" collecting boat, who collected and identified the fish, and especially to Mr. Donald Zumwalt, who permitted the study on this boat; and to Trinity Christian College (Palos Heights, Illinois) for partially financing this survey.

SKIN INFECTIONS CAUSED BY INOCULATION OF

MYCOBACTERIUM MARINUM FROM AQUARIUMS

J. Walter Wilson, M.D.*

Aquarium hobbyists should be aware of the possibility of acquiring a certain troublesome, exceedingly persistent, chronic type of bacterial skin infection in injuries sustained from handling aquariums or their contents. Although cases probably of this nature have been occasionally reported during perhaps the last half-century, it is only because of progress in bacteriology during the past 15 years that the disease has been clearly documented and its causative organism cultured and conclusively identified.

The ever growing popularity of aquarium keeping has increased the frequency of this infection. However it has not yet become sufficiently common to be suspected at first inspection by more than a small percentage of doctors other than skin specialists, and even they will be likely to consider several other diseases more probable and test for them. Thus the correct diagnosis may not be made until extensive laboratory studies have failed to confirm some other more commonly encountered disorder. Such a delay can be avoided in many instances if the history given to the doctor during the first visit includes the fact that aquariums or their inhabitants have been rather constantly contacted. At this point even a physician who has had some experience with this infection may like to consult (what at the time of this writing is) the latest article on the subject, by Adams of Stanford and his co-authors, published in the Journal of the American Medical Association, January 19, 1970, (Volume 211, page 457). This can then serve as an excellent guide to the laboratory tests needed to make the diagnosis. This infection is discouragingly slow to respond to all combinations of treatment thus far employed, and without a definitely established diagnosis it is difficult to maintain sufficient patience on the part of the infected person as well as the doctor to persist long enough to succeed. Otherwise all too often several weeks may be devoted to each of two or three wrongly selected treatment schedules before the right course is found.

---

*Chief, Section of Dermatology, Veterans Administration Hospital, Long Beach, California; and Clinical Professor of Dermatology, University of California at Irvine.

The patient should be alerted to the possibility of this oddity if a finger, a hand, or an arm has sustained an injury sufficient to break the skin, either by direct contact with some sharp or abrasive object in (or associated with) an aquarium, or similarly acquired elsewhere and subsequently exposed to such materials. Almost all such lesions will, of course, heal completely in a week or so in the usual manner. If a different, persistently delayed course is followed, an unusual type of infection must be suspected and the possibilities should include the one under consideration.

Typically a firm, raised, red, mildly tender lump appears at the point of injury, and slowly enlarges during the succeeding several weeks, both in diameter as well as in height above the level of the surrounding normal skin. Sometimes the central part of this swelling becomes ulcerated and discharges pus. Many of the patients develop a chain of similar lesions, somewhat separated, but joined by reddened streaks, and extending from the original one upward toward the elbow or armpit, and often there are tender, swollen lymph glands even in these distant areas. This sort of linear extension is considered to indicate that this is probably the first infectious contact that that particular person has ever had with this bacterium, or possibly even with any of its close relatives. Conversely, if the infection remains strictly localized to the injured region, it seems likely that this is due to previous contact with the same, or similar bacteria, having taught that particular person's body how to resist it more efficiently.

The causative microbe belongs to a group of organisms possessing certain features somewhat resembling fungi, and hence named <u>Mycobacteria</u> (funguslike bacteria). They also have the ability to retain certain colored dyes, (called "stains") even after being acted upon by acidified solutions, and are therefore termed by bacteriologists "acid-fast". Some of these bacteria can cause disease in animals and some cannot. The causative organisms of tuberculosis and leprosy belong to this group, and were, of course, long ago established as "pathogenic" (able to cause disease) because of the huge numbers of persons who were easily shown to be thus infected. Other species have become similarly established, as pathogenic, but only after much more experience, slowly accumulating during many years because of the comparative rarity of actual infections which they succeed in causing. As a group within the main group these are referred to as the "atypical" <u>Mycobacteria</u>.

Within this sub-group the various species are separated principally because of differences in their behavior in laboratory cultures. Some grow very rapidly, others slowly; some cannot produce color, some can do so, but only when grown in the light, and some succeed even in the dark. The one we are interested in here needs to grow in the light in order to produce color, and then it makes an orange pigment. It also does not like to grow when incubated at 37^{O}C (98.6 F.) which is the internal temperature of the human body. This is fortunate and important, obviously being one of the factors preventing its infections from involving internal organs. This is in sharp contract to the bacterium of tuberculosis (Mycobacterium tuberculosis) which, of course loves to involve the lungs, and other deep seated areas of the body. It is interesting to note that the organism which causes leprosy (Mycobacterium leprae) also does not like to extend deeply, limiting its effects mostly to the skin and superficial nerves, although we have not succeeded in growing it in culture in glass vessels so as to prove that its temperature choice is the determining factor.

Looking backwards, from our level of present day knowledge, it is likely that a case reported by Cobbell in 1918 was caused by this bacterium. However it was not until about 1950 that it was discovered that persons frequently acquired an unusual type of chronic infection, usually on elbows, knees or ankles because of injuries sustained from contact with sand or rough concrete while climbing out of the water of swimming pools. First in Sweden, a little later in Canada and the U.S.A. large groups of patients were shown to have developed these sores, which were called "swimming pool granulomas". When it was cultured and studied bacteriologically, the causative bacterium was found to belong to this group and was named accordingly "Mycobacterium balnei" (balnei = of bathing).

It was Sheldon Swift and Harold Cohen (also in California) who first reported in 1962 that similar infections could be acquired from aquariums (New England Journal of Medicine, Volume 267 page 1244), and it soon became established that this was the same bacterium as that acquired from swimming pools. However, soon it was also shown to be identical with an organism which had previously been recovered from other aquatic sources and animals, particularly from the sea and considered to be causing "fish tuberculosis", and therefore named Mycobacterium marinum. This is now the accepted species designation, because of botanical priority as well as because it seems more appropriate than "balnei" which implies that it should be limited to direct relationship with bathing.

It requires only a little reflection to understand that tropical fish aquariums and heated swimming pools are carefully maintained at the very temperature level which this bacterium prefers - that is, about 70 to 75° Fahrenheit (21 - 24° Centigrade). Therefore, they can easily become huge cultures of such bacteria. Of course in well chlorinated and filtered swimming pools this cannot occur, but aquariums are almost ideally suited, particularly if they are allowed to become oversupplied with organic materials from food and animal or plant residues. Some of the early clues were brought to light because of infection acquired in cleaning up after wholesale death of all life in an aquarium had occurred. Unless such an event can be obviously explained, for example by a defective thermostat, it is advisable to protect one's hands while correcting the tragedy by using rubber gloves and being extremely cautious to avoid all possible skin injuries. Subsequently, either chlorination, or permanganate sterilization should be allowed time enough to be effective, before the tank is used again.

I have personally observed, during recent years, seven infections proved to be of this nature, and I am convinced that I sustained a similar infection over 20 years ago, much before I or the medical profession in general possessed enough knowledge to care for it scientifically. The particular organism involved in causing this infection is not willing to cease its activities because of the influence of any of the drugs which have proved to be so effective in fighting tuberculosis. Iso-nicotinyl-hydrazide (INH) has been given to many patients, and may have helped a little, but never dramatically. Streptomycin and PAS (para-amino-salicylic acid) have been equally disappointing. Synthetic vitamin D seems to be useless.

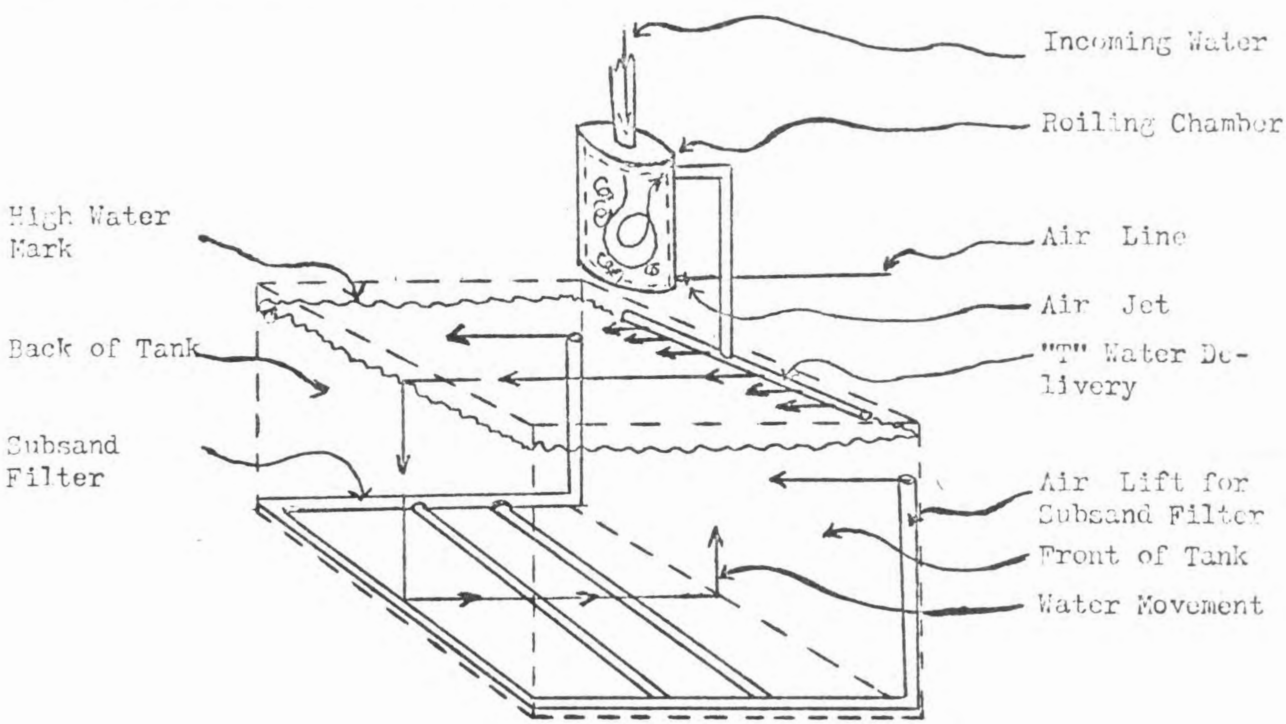
Most cases have indicated that the ultimate deciding factor is the ability of the normal human body to learn how to resist the disease by developing specific immunological resistance, which is, after all, the most important of all of our defenses. However, I am convinced that if the lesion is brought to the attention of an experienced doctor while still small, and while still localized to the region of the injury, a week or ten days may suffice to confirm microscopically the likelihood of this being the correct diagnosis. In such a case, complete eradication of the original small area by using a heated cautery or electric spark machine seems preferable to waiting until the infection spreads to a larger area. In the meantime careful cultural studies can be carried out, and further appropriate care outlined.

A NEW AERATION SYSTEM DEVELOPED BY THE WAIKIKI AQUARIUM STAFF

Frank Sutherland
Aquarist, Waikiki Aquarium

The Waikiki Aquarium has an open water system with the sea water drawn from a well extending approximately 85 feet deep into fossil coral reef. The water filters through sand and coral strata which rids it of most floating matter: aerobic bacteria which occur in the sand, and physical oxidation break down organic detritis and most disease organisms. By the time the water reaches the well it is crystal clear and relatively disease free. However, due to the consumption of O_2 by the bacterial and physical oxidative processes the O_2 content of the water is extremely low. The saturation concentration of dissolved oxygen in the open sea near the Aquarium has been determined to be approximately 7 ppm; oxygen tests show the well-water to be less than 1 ppm. No aeration of well-water is provided prior to delivery to individual display tanks. Economic constraints preclude the installation of an aeration tower in the immediate future. Air stones placed tanks increase the O_2 concentrations to 5 ppm (approximately 70% saturation) but this is, at best, borderline for maintaining healthy, long-lived reef fish that are acclimated to inshore sea water which is saturated with O_2. Airstones are used to achieve a 5 ppm concentration but the air must be turned up so high that the tank water usually becomes impregnated with tiny, high pressure air bubbles. Not only does this diminish water clarity, but the bubbles have been linked to a form of the disease popeye.

SCHEMATIC OF AERATION AND "T" WATER DELIVERY SYSTEM



The Aquarium staff has developed a simple and effective solution to the problem which may be of use to other aquarists. Instead of allowing the incoming water to flow directly into one of the tanks, as is the usual method, a small chamber was constructed above the tank into which the water initially flows. The chamber was fitted with an air jet in the bottom with the jet open full giving the poorly oxygenated water a strong roiling to provide O_2 absorption at the maximum rate. The water then flows out of the chamber into a "T" arrangement which is situated in the front of the tank at the high water mark. Small holes were drilled in the "T" on the side facing the rear of the tank; this releases the incoming aerated water in small streams across the surface when the tank is full. The velocity of the incoming water causes it to flow in a layer at the surface of the tank from front to rear providing prolonged exposure of the water to the atmosphere, thus facilitating more O_2 absorption. A subsand filter was installed in the tank in such a way that the water flow from the air lifts augments the front to rear flow of the incoming water.

Tests in the tank for which this system was designed have shown the water to be 100% saturated with dissolved oxygen; also, no unsightly high pressure air bubbles are released into the tank eliminating the popeye hazard, and good water circulation is maintained which insures effective distribution of the oxygenated water.

Pteropod: Clione sp. "Flying snail" Approx. 4 X

Photograph by Charles Eames, Venice, California

AMMONIUM CHLORIDE AND BIOLOGICAL FILTRATION

Stephen D. Walker
San Antonio Zoological Gardens and Aquarium

In the preparation of closed-system marine aquariums, by far the most important consideration is the proper establishment of nitrifying bacteria on the biological filter. Yet the functions and characteristics of these bacteria are still often confused and misunderstood. This misunderstanding has led many amateurs (and others who should know better) to lose specimens to that terrible beast, "the new tank syndrome". The new tank syndrome is nothing more than a mistake on the part of the aquarist in not giving the biological filter enough time and the proper substances to become prepared for handling animal by-products. One easy and efficient method for supplying these necessities is the subject of this paper.

First we should have a basic understanding of the bacteria themselves and the services they perform in a closed-system aquarium. In essence, the presence of these bacteria is needed to rid the tank of ammonia. The non-ionized form of ammonia (NH_3) is excreted mainly through the gills of fishes and is a real killer in marine aquariums in even small amounts (0.5 parts per million). There are two genera of bacteria useful for getting rid of the ammonia, these being Nitrosomonas and Nitrobacter. Nitrosomonas takes up the ammonia and, through its life processes, converts it to nitrites, which are still semi-toxic but very much less so than ammonia. The nitrites now dissolved in the water are then taken up by the bacteria Nitrobacter, which converts them to nitrates. Nitrates are essentially harmless even in large concentrations. Algae are then able to use the nitrates and remove them from the water; thus, an equilibrium is established ridding the tank of ammonia and providing food for algae, which in turn may become food for fishes, and so on.

Now we turn to the practical applications of establishing and maintaining these beneficial bacteria in sufficient quantities. First, the autotrophic bacteria described above are aerobic and, thus, require great amounts of oxygen to perform their conversions. The simplest and least expensive method of providing this oxygen is through the use of undergravel filters. The bacteria attach to the outer layers of the gravel, and the undergravel filter pulls oxygen rich water around them. Previous research indicates that a substrate with a grain size of 2-3 mm, and a depth of 5-6 cm is the most efficient. The problem of culturing the bacteria in sufficient amounts now arises. One common method is to innoculate the tank with gravel from an established aquarium and, then, add a few hardy animals to supply the ammonia for the growth and reproduction of these bacteria. Research from

Japan, however, indicates that only about 10% of the bacteria established on the filter bed by this method are of the beneficial type, while the rest are heterotrophic organic eaters and others even less desirable. The organic wastes also produced by the fishes feed the heterotrophic bacteria pushing the autotrophs out. Moreover, it has been shown that organic matter itself inhibits the reproduction of the beneficial autotrophs. Therefore, if ammonia alone could be supplied, the autotrophic bacteria should become established on a much greater proportion of the filter. Research at the Case Western Reserve University indicates the use of ammonia producing substances would perform this function. We at the San Antonio Zoo Aquarium have been experimenting with one of these substances, and our initial findings are interesting.

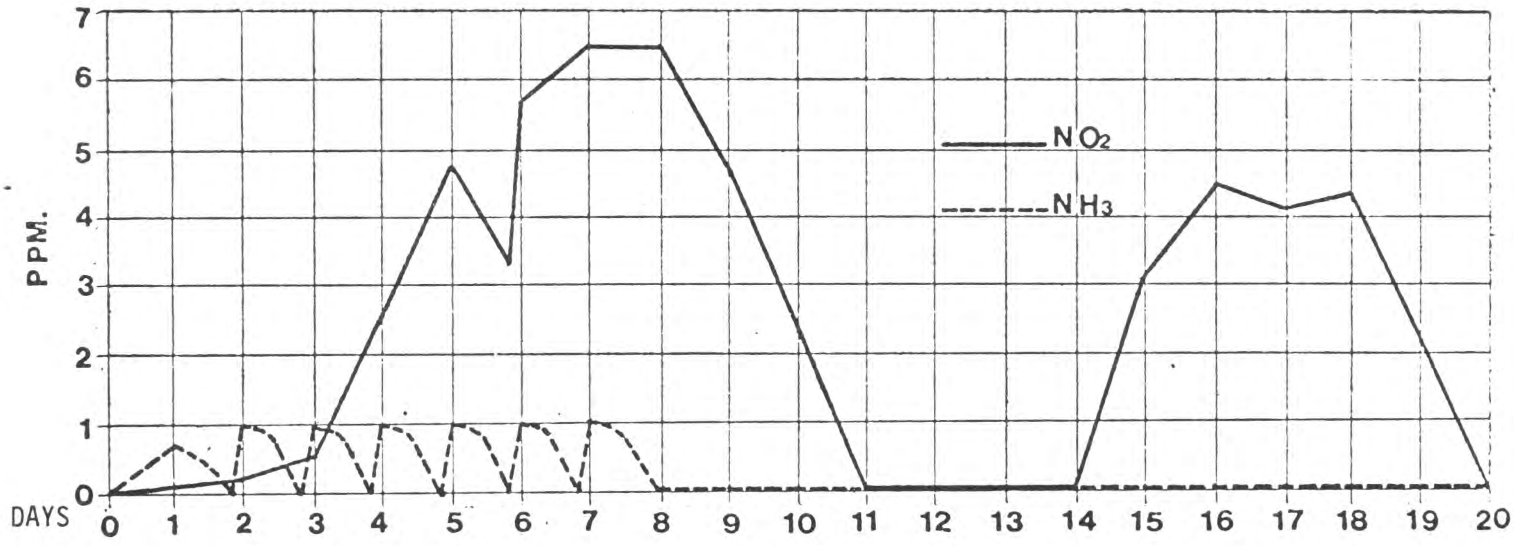
For the test, a 50 gallon, all-glass aquarium was set up with a Halvin undergravel filter and a 6 cm layer of dolomite filter material. The aquarium was then innoculated by introducing a handful of gravel from a large tank which had been operating for some time. "Instant Ocean" synthetic marine salt was used at a specific gravity of 1.025, and the temperature varied between 73° and 80°F.

The inorganic ammonia source used in the test was ammonium chloride. This substance breaks down in water to form ammonia at a rate of 1 ppm for every 3 ppm of ammonium chloride added. In practice, a stock solution of 6% (by weight) ammonium chloride in water was added to the test tank at a rate of 1 cc/5 gallons daily for seven days. This gives a concentration of approximately 1 ppm at each addition or 7 ppm total for the test. The solution is given daily instead of in one dose, because a large concentration of NH_3 has shown to be a deterent to the reproduction of Nitrobacter bacteria. To prepare the stock solution, it should be remembered that 1 ml of distilled water weighs 1 gm; therefore, 6 gm of NH_4Cl per 94 ml of water gives the 6% solution.

Tests were conducted each day for three weeks on the tank. These tests included ammonia, nitrite, and pH, and were performed twice daily for the first week (before and after addition of NH_4Cl) and once daily after the conclusion of the additions. The results are given on the graph that accompanies this article.

These results are those which would be generally anticipated but with some interesting variations. The ammonia level never exceeded 1 ppm, and in fact, fluctuated back and forth from 0 to 1 ppm with each 24 hour's addition. The nitrite level, on the other hand rose quickly and steadily to a maximum of 6.4 ppm on the 7th day. It then began to decline slowly, until between the 9th and 11th days when it dropped from 4.8 to 0.0, which is rather unusually fast. It appears, then (as has been said by previous researchers), that the Nitrosomonas nitrite producing bacteria become established much more rapidly than the nitrate producing Nitrobacter.

Unexpectedly on about the 15th day, the nitrite level again began to rise. It remained high for five days and then began to decline and has remained at zero ever since. We have proposed this rise to be the result of the growth of de-nitrifying bacteria which convert nitrates back into nitrites. This would certainly seem probably because of an expected large amount of of nitrates present before enough algae could grow to complete their job of absorbing the nitrates. Once the tank was innoculated with algae, and it began to grow under 24 hour flourescent lighting, the nitrates did fall to zero again. Unfortunately the nitrate level was not monitored, and therefore this de-nitrifying action is more or less conjecture on our part.



The values of nitrite and ammonia levels versus time (in days), with the use of Ammonium chloride as an ammonia source for nitrifying bacteria. The rise and fall of nitrite follows the ammonia fluctuations as Nitrobacter bacteria are appearing after the growth of Nitrosomonas bacteria. The second nitrite rise is probably due to the growth of de-nitrifying bacteria. It should be noted that the nitrite and ammonia values are given as total Nitrite-nitrogen and Ammonia-nitrogen respectively and have not been converted by use of correction factors. In other words, these values are those read directly from the test kits.

According to the Case Western Reserve University study, a 290% increase in nitrification rates can be expected when a tank is set up in this fashion, and herein lies the beauty of it all. With this increase, almost three times as many animals could conceivably be cultured. One must also take into consideration oxygen consumption and sufficient space for some of the territorial creatures, but still this appears to be a major breakthrough in marine aquarium keeping. We at the San Antonio Zoo Aquarium are quite impressed with the speed, ease, and low expense involved in this procedure, and are planning further studies into its characteristics and applications.

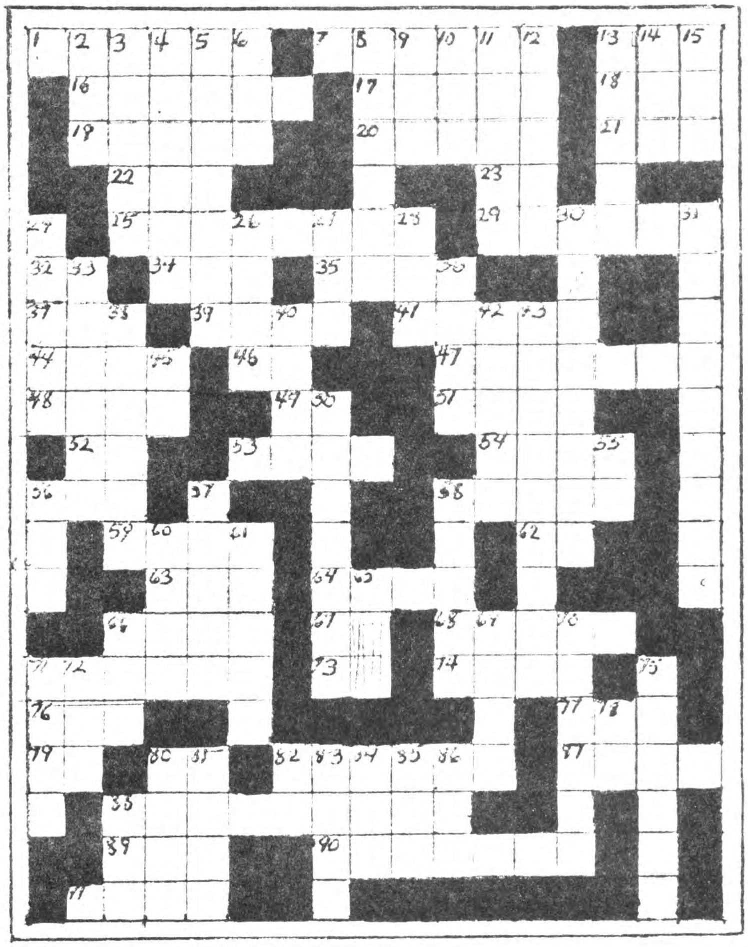
REFERENCES

Kawai, A., Y. Yoshida, and M. Kinata. Biochemical Studies on the Bacteria in Aquariums with Circulating Systems. II. Nitrifying Activity of the Filter Sand. Bull. Jap. Soc. Fish. (1965) pp. 31, 65-71.

Siddall, Scott E. "Recent Research in Biological Filtration", The Marine Aquarist. Vol.4, No.1. (1973) pp. 6-12.

Spotte, S. H. Fish and Invertebrate Culture. John Wiley and Sons, Inc. (1970) pp. 3-21.

FISH FANCIERS
CROSSWORD



FISH FANCIERS CROSSWORD

ACROSS--

1. Eel or typewriter need
7. Baby or centropomid fishes
13. Gadus
16. One time herring genus: C----- (Artedi) Linnaeus
17. Type of predators that attack fish from above
18. Small fish used for tuna bait- Praenesus insularum - Hawaiian
19. Newly collected fishes can prove alarming since they --- -- little
20. Pelagic sea squirt genus
21. Sir (abbr., pl.)
22. Labor union (abbr.)
23. All right
25. Water lily, pond lily (French)
29. Subgenus used by Jordan and Everman for 3 pickeral species.
32. Alternative
34. In the absence of better preservatives fish have even been preserved in ---.
35. Giant needlefish, Strongylura gigantea (Hawaiian)
37. Common name for one type of snapper
39. The advanced aquarist must never risk the consequences of feeling too ---- in his or her achievements
41. Common name for one barracuda
44. Genus of the bowfin (calva)
46. 51 in Roman numerals
47. Brought to bear; ------ influence
48. Diving bird with mottled plumage
49. University of California (abbr.)
51. Prefix, self
52. Road (abbr.)
53. Deep, slimy mud
54. Narcotic
56. UN organization on fisheries, etc.
58. State your intentions again in some card games.
59. Goby or tetra
62. Like
63. Longest division of geol. time: one or more periods.
64. Unicorn tang, Naso unicornis (Hawaiian)
66. Slender
67. Initials of late Supt/Curator of Steinhart Aquarium
68. Gobiesox strumesus is a ----- fish
71. Rainbow, clown or slippery dick
73. Egyptian sun god
74. Sturgeon or beluga found in the Aral-Caspian
76. Wrasse, Lachnolaimus maximus: --- fish
77. Last name of biologist in U.S. who bred and raised the clownfish
79. For example (abbr.)
80. Pronoun
82. Labrid of N. Atlantic: blackfish, oysterfish or ------.
87. Pike genus
88. Dir. of Coral Reef exhibits & SWA magazine (last name)
89. Balaenoptera borealis, --- whale
90. Northern and southern lights
91. Exotic fishes resemble precious stones, their colors sparkle like living ----

DOWN--

2. A type of fishing practiced from temperate to polar regions
3. Skin sore; blister or blotch
4. Spread or fish
5. Toadfish genus (beta, phobetron, etc.)
6. Prefix for new or recent
8. Type of grouper found in tropical SW Atlantic, Epinephelus striatus
9. Eggs
10. Fish or black gold
11. Silky fiber used for filling life preservers and pillows.
12. Eel, fish or reptile
13. "Lake herring" or _____, whitefish family member, Coregonus artedii
14. Paddle or fish
15. Back (French)
24. Brain, finger or banded shrimp
26. University of Miami. Institute of Marine Sciences. Marine Laboratory (Abbr. used on fish collection labels)
27. Jawless fish or witch
29. N. Pacific fishes with extreme flexibility or something you throw in - if you give up.
30. Minnow or shiner genus (petersoni, hypselopterus)
31. Genus of sergeant major and night sergeant
33. A shark's companion
36. Type of biological waste
38. Porcupine and baloonfish genus
40. Left-handed flounders (Bothus) known as Paku and ____ to Hawaiians
42. Morays, wrasses and parrotfishes _____ much slime or body mucous
43. Ray genus of eagle ray (narinari)
50. Scianid fish with resonating swim bladder chamber (e.g. D & _)
55. Editor (abbr.)
56. Some fishes even have the ability to ___ (Exocetids)
57. Genus of colorful wrasses (gaimardi, julis, angulata, etc)
58. Offensive household pest or fish (Rutilus rutilus)
60. Elongated fishes: f.w., marine, anadromous, catadromous.
61. AFS list of fishes gives common and scientific _____
65. Exclamation of sudden discovery or Hawaiian name when repeated twice for the needlefish, Ablennes hians
66. Droop
69. Fishes that form mud cacoons or something a diver can use
70. Genus of man-of-war fish (gronovi)
71. Type of dasyatid ray
72. Eggs or egg-laden ovary of fish
75. Petty Officer in the U.S. Navy or old time free-holder farmer in England
80. Single unit on a list
81. Type of buffer: abbreviated name
82. You (French or Italian)
83. Culture medium
84. Parrotfish (Hawaiian)
85. Gob or sailor
86. Wahoo (Hawaiian)
88. Compass directions