OODINIOSIS IN THE GULF OF CALIFORNIA:
A CRITICAL REVIEW OF ITS TREATMENT

by

Norman Donald Morrison III

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STATEMENT BY AUTHOR

SIGNED: Norman L. Morrison III

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Donald A. Thomson
DONALD A. THOMSON

August 25, 1967
Date

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ABSTRACT

A small race of *Oodinium ocellatum* comparing favorably in all aspects except size to the species description was found to be ectoparasitic on *Quietula guaymasiae* and other fishes collected at Puerto Penasco, Sonora, Mexico. The symptomatology of oodiniosis revealed only one pathognomonic character, a reddish-pink mucoid secretion at the base of the pectoral fins. Other symptoms were non-specific for oodiniosis, being indicative of a general diseased state. Treatment with several chemotherapeutic agents showed varying degrees of success. Selected copper salts tested for their stability in aquaria containing a glass substrate as contrasted to a calcareous sand substrate showed a slight increase in stability with increasing molecular weight. The presence of calcareous material substantially reduced the stability of copper ion. Copper sulphate applied directly at Cu^{++} concentration of 0.2-0.4 ppm was found to be the most practical method of treating oodiniosis.

CHAPTER I

INTRODUCTION

Oodiniosis is a highly infectious ectoparasitic disease of teleost fish and aquatic amphibians. Freshwater oodiniosis is caused by two species, *Oodinium limnecticum* Jacobs, 1946, and *Oodinium pillularis* Schaperclaus, 1951. Marine oodiniosis is caused by a single species, *Oodinium ocellatum* Brown, 1931. *Oodinium ocellatum* is a naked dinoflagellate of the Family Blastodiniidae which represents a multigeneric family parasitic in or on a wide variety of animals.

Brown (1931) first described a parasitic dinoflagellate, *Oodinium ocellatum* occurring on the general body surface of tropical marine fishes imported from the East Indies and the West Indies, in the Aquarium of the Zoological Society of London. In later studies Nigrelli (1936) described infestations of *Oodinium ocellatum* from Sandy Hook Bay, New Jersey to Key West, Florida. Dempster (1955) recorded oodiniosis in the tanks at the Steinhart Aquarium in San Francisco. This epizootic was believed to have resulted from importation of large shipments of tropical reef fishes from the Hawaiian Islands. Dempster also discovered moderately severe infestations in several indigenous

Californian coastal fishes, namely striped bass (Roccus saxatilis), rubberlip seaperch (Rhacochilus toxotes), and lingcod (Ophiodon elongatus). The latter findings represent the first discovery of Oodinium ocellatum in nonmigratory fishes native to cooler temperate waters.

Early in 1966 several major outbreaks of a protozoan parasitosis causing high mortalities occurred in aquaria containing fishes from the upper Gulf of California at the University of Arizona. Since the epizootics resembled oodiniosis, investigations were initiated to: (1) identify the causative organism; (2) describe the symptomatology of oodiniosis in the estuarine gobiid fish Quietula guaymasiae; (3) re-evaluate some commonly employed chemotherapeutic agents applied as dips, baths, and prolonged bath treatments; (4) analyze the comparative precipitation rate of copper ions from selected copper compounds in sea water; and (5) determine the effect of a calcareous sand substrate on the copper precipitation rates.

CHAPTER II

MORPHOLOGY AND TAXONOMY

According to Nigrelli (1936), the parasitic stage of O. ocellatum is a pyriform organism which has a mean length of 60.1 microns and a mean width of 50.1 microns. The organism adheres to the host's surface tissues by means of a delicate system of rhizoids arising at the posterior aspect from a thickened retractile peduncle, which extends through an orifice in the cellulose cell wall. Also attached to the peduncle is an elongated ribbon-like flagellum which can be seen slowly sweeping over the host's epidermis. Immediately internal and slightly lateral to the peduncle a stigma or eye-spot is present. This organelle consists of a broad red bar and a slender black pigmented bar containing a clear refractile area between the two bars. The internal structures consist of a centrally located nucleus ranging in size from 12 microns to 30 microns. Distally surrounding the nucleus are found numerous pale green chromatophores, which upon treatment with iodine-potassium iodide yielded a characteristic purplish color, thus indicating the presence of starch.

Once the adult cyst has been removed from the host the rhizoids, peduncle, and flagellum are retracted and the cell wall orifice is gradually closed by a cellulose secretion. After the orifice has closed cellular fission commences. The divisions are more or less equal and continue to the 128 cell stage whereupon dinospore metamorphosis occurs. After flagellation has occurred, the palmella undergoes one more division yielding 256 motile dinospores. The dinospores undergo several minor maturation transformations whereupon they assume the typical free-swimming dinoflagellate form. In the metacyclic dinoflagellate the epicone is slightly smaller than the hypocone. The nucleus has the typical adult morphology. The ocellus is located to the right medial aspect of the epicone. The free swimming dinoflagellate stage of _O. ocellatum_ is a small organism measuring approximately 12 microns in length and 8 microns in width.

The identification of the species of _Oodinium_ occurring in the upper Gulf of California was based on the life cycle, on the morphology, the size of the adult parasitic stage on various tissues of _Quietula guaymasiae_, and the free-living dinoflagellate stage. Natural infestations were discovered in freshly caught _Quietula guaymasiae_. In 20 fish examined, mild infestations ranging from 1 to 5 cysts per fish were found on the pectoral fins and gills. The life cycle of the species from the Gulf of California compared favorably to the life cycle as given by Nigrelli (1936).

The morphological characteristics of the parasite upon which identification was based, as shown in figures 1 and 2, were:

1. A caudal peduncle terminating in few rhizoids.
2. An elongated ribbon-like undulating membrane.
3. An eye-spot (stigma neuro-motor complex) consisting of a black and a red pigment bar with a highly refractile area in between.
4. The large round to ovoid centrally located nucleus.
5. The pear-shaped contour of the adult parasite.
6. The presence of peripheral green chromatophores yielding the characteristic purplish starch reaction with iodine-potassium iodide.
7. The presence of free-swimming dinoflagellate stage with an ocellus in the right aspect of the epicone.
8. The presence of palmella stages in the life cycle.

The adult size based on 50 measurements was 50.2 microns in length and 39.7 microns in width, which was considerably smaller than Nigrelli's measurements of 61.1 microns in length by 50.1 microns in width.

The free-living dinoflagellate stage conformed in all details, except size, to the description given by

Fig. 1

Unstained *Oodinium ocellatum* attached to the pectoral fin of *Quietula guaymasiae* showing stigma (st.), peduncle (pd.), nucleus (n.), chromoplastids (ch.), and cellulose membrane (c. cw.). Magnification 860 X.

Fig·1

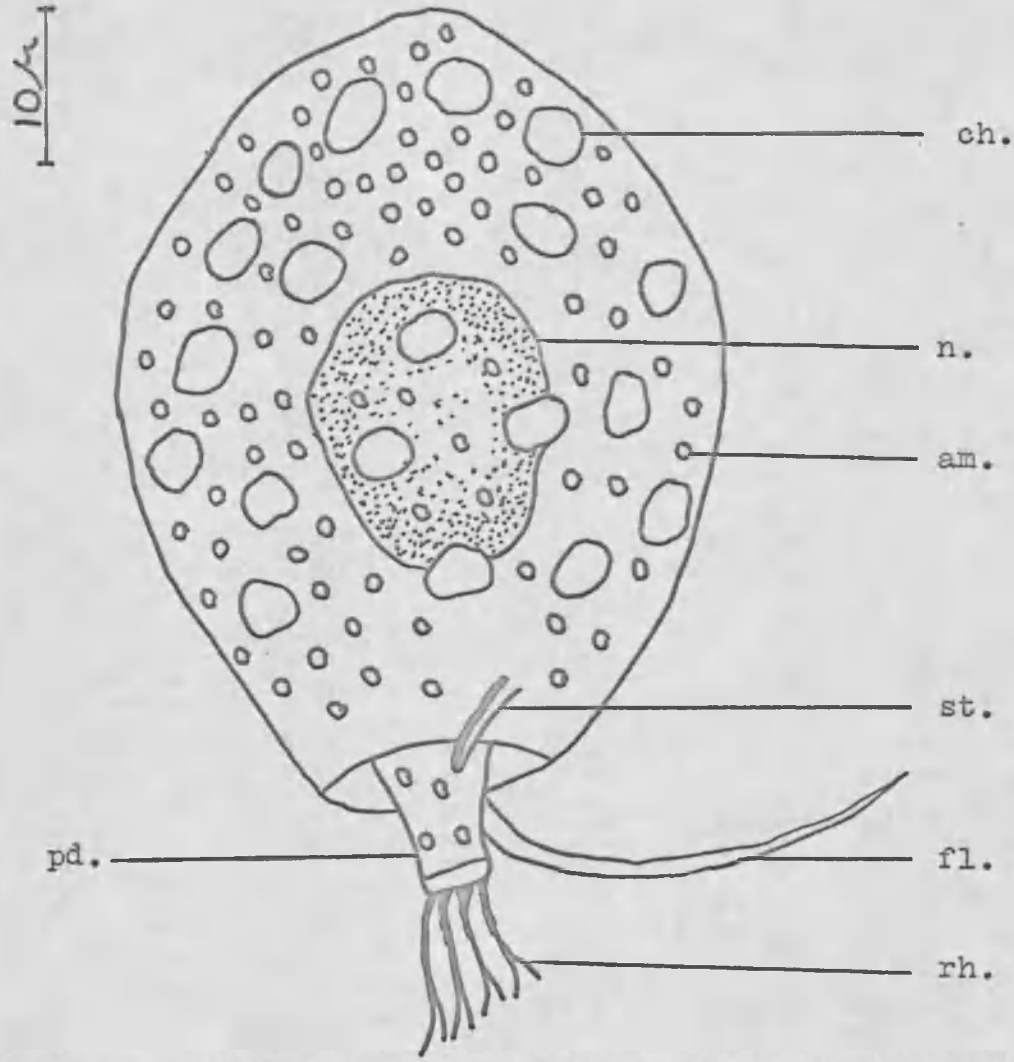

Fig. 2.--Parasitic stage of <u>Oodinium</u> <u>ocellatum</u> showing major morphological features. am., amyloid granules; ch., chromoplastids; fl., flagellum; n., nucleus; pd., peduncle; rh., rhizoids; st., stigma-neuromotor complex. (Modified after Nigrelli, 1936)

Nigrelli (1936). The dinospore size based on 20 measurements, was 9.2 microns in length and 6.5 microns in width, which was smaller than Nigrelli's measurements of 12 microns in length and about 8.0 microns in width.

Except for the size differential, all other features were in accordance with Nigrelli's description. Therefore, the parasite was determined to be a small race of *Oodinium ocellatum*.

CHAPTER III

SYMPTOMATOLOGY

The subject of *Oodinium ocellatum* has been investigated by several authors; however, the general symptomatology in fish has been neglected in all papers. Nigrelli (1936), in his extensive research on the morphology and life cycle of *O. ocellatum*, only casually mentioned one symptom--a pinkish cast in the mucous secretions. Dempster (1955) stated two major symptoms; namely, increased respiration and the tendency for the fish to congregate near the surface. Reichenbach-Klinke and Elkan (1965), in their review of the main symptoms of fish disease, attribute only two symptoms to oodiniosis: (1) a velvety surface, which is characteristic of *O. limneticum* and *O. pillularis* and (2) permanently opened operculum which is characteristic of all three species parasitic on piscine hosts.

The symptomatology of oodiniosis in the goby *Quietula guaymasiae* and also in the related gobiid fishes (*Gillichthyes spp.* and *Ilypnus spp.*) follows a rather characteristic pattern of events. The majority of symptoms are variable and may be considered to parallel closely the anoxia syndrome or ectoparasitism by other organisms of diverse phyla. The only symptom characteristic of oodiniosis is a reddish mucoid secretion on the proximal aspects of the pectoral fins and to a lesser degree on the pelvic fins.

The earliest symptoms of disease, which occurred from 24 to 36 hours after inoculation of the aquarium with peridinian dinoflagellates, consisted of scratching against the substrate and occasional bursts of erratic swimming. The fish, which normally rest on the substrate, periodically engaged in short bursts of twisting gyrations lasting from one through 4 seconds, whereupon they returned to a normal resting position. According to Reichenbach-Klinke and Elkan, (1965), the gyrations are interpreted as an attempt to rid irritating parasites attached to the skin. As the infestation progressed, the gyrations increased in magnitude, duration, and frequency until approximately 48 hours prior to death, at which time swimming became progressively erratic and uncoordinated.

Another generalized indicator of disease, which appeared 2 to 4 days post-inoculation, was the depression of the dorsal fin against the body surface. Except during periods of swimming, the dorsal fin remained depressed almost constantly until death.

The most obvious pathological symptom was a progressive anoxia. The first indications of anoxia were increased opercular movements; these increased from a non-infected basal rate of approximately 50 beats per minute to over 115 beats per minute in massively infested fish near death. The anoxia was manifested in a progressive tendency to seek areas of higher oxygen concentrations, that is, areas near the air-water interface. At first the fish would either swim to the surface for a few seconds or attach themselves by means of the modified pelvic fins to the glass walls of the aquaria within a few centimeters of the surface. Later, 24 to 48 hours prior to death, the fish remained almost constantly attached to the aquaria walls at the air-water interface. At this stage, the opercula were markedly swollen and there was a reddish-pink cast to the mucoid secretions on the surface of the pectoral and pelvic fins. From 2 to 15 hours prior to death, the fish lost almost all coordination and just floated in a position vertical to the surface. An air bubble formed in the oral cavity and pharyngeal region, which pulsated with each opercular movement. Periodically the fish passively sank, tail first, to the substrate remaining on the substrate in abnormal positions for varying lengths of time, whereupon they would attempt to return to the surface or expire upon the substrate. In all cases the mortality was complete for the entire fish population in the infected aquaria.

Immediately after death, an autopsy was performed on each fish to ascertain the number of cysts present on selected areas, namely the gills and the pectoral, pelvic and caudal fins. The results in Table 1 show a definite preference for certain tissues. In each case the measurements were based on a microscopic field of 1.075 mm^2 and two samples were examined from each tissue on each of 40 subjects. Figure 3 illustrates the cyst density on the pectoral fin of Quietula guaymasiae.

Fewer cysts were present on those tissues in which contact with the substrate was greatest, namely, the caudal and pelvic fins. In almost every case the caudal and pelvic fins were in almost constant contact with the substrate and therefore were subject to frictional abrasion. In contrast, the pectoral fins were commonly held vertically with only the most ventral areas in actual contact with the substrate. Due to respiratory action, water is constantly being circulated over the gill and pectoral fin tissues, this respiratory action increases the number of dinospores coming in contact with these tissues. The gills, being protected from abrasion, offered the most protected habitat and this was illustrated by the fact that the vast majority of parasitic cysts were found in this area. Another factor, though not substantiated, is that the gill tissue has the greatest blood flow therefore the best nutritional environment for the developing parasitic cyst.

Fig. 3

Iodine stained *Oodinium ocellatum* cysts attached to the pectoral fin of *Quietula guaymasiae*. Note the parasite aggregations in the inter fin ray space and the trematode metacercarial cyst attached to the fin ray. Magnification 125 X.

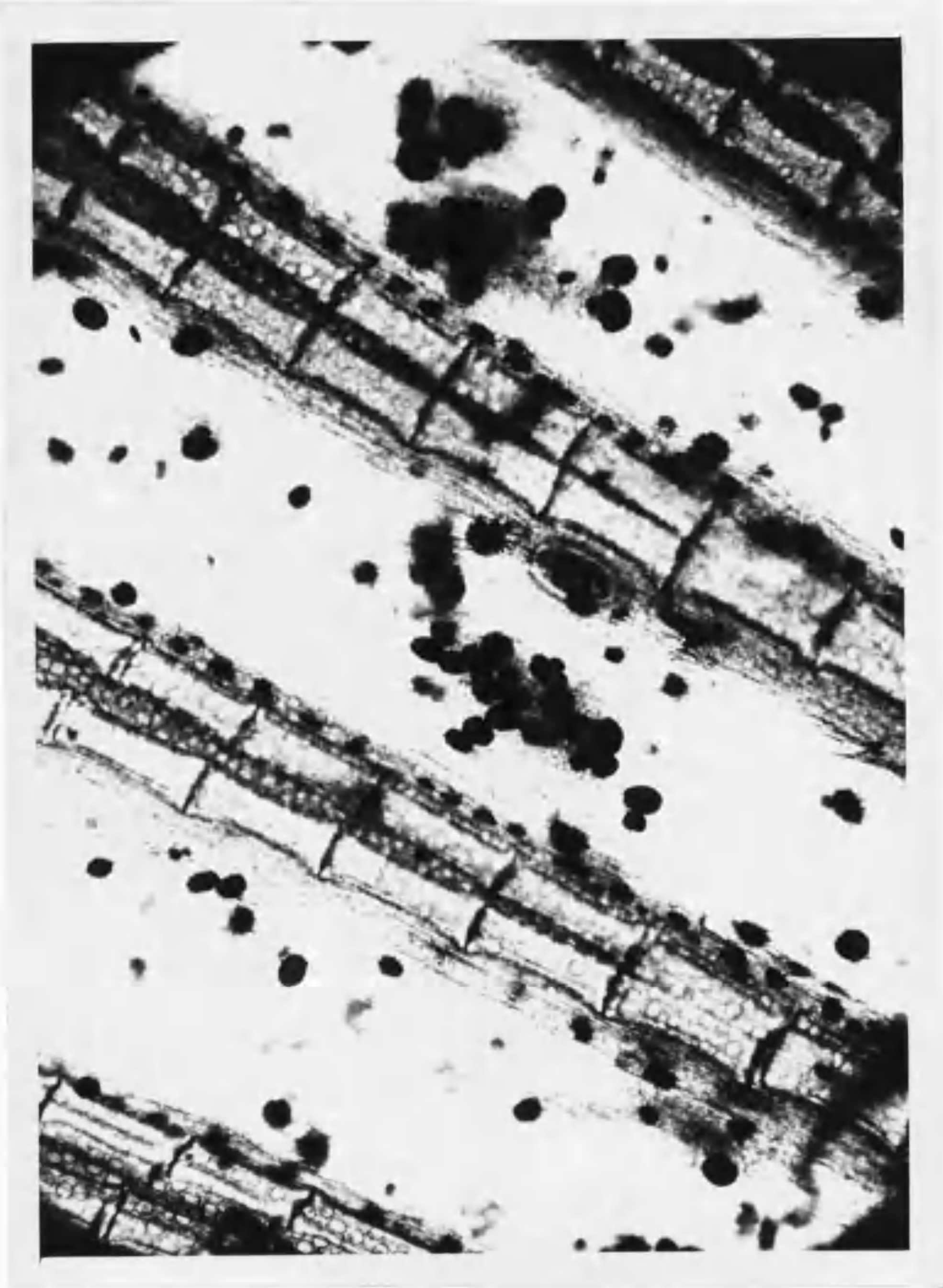

Fig. 3

Table 1

Cyst density of *Oodinium ocellatum* per unit area ($1.075\ mm^2$) on various tissues of *Quietula guaymasiae*

Tissue	Ave. # cysts	Range	Standard deviation
Gill tissues	110	30-200	46
Pectoral fins	65	23-170	35
Pelvic fins	22	7-60	17
Caudal fins	26	5-57	16

Post-mortem examination of the gill tissues revealed pathological alterations. The tissues were pale red rather than the typical deep red color. The filaments were stunted and edematous, lacking the distinct filament outlines present in normal gill tissues.

Although the exact cause of death due to oodiniosis is not known, the major pathological symptom, i.e. progressive anoxia, indicates the probable mechanisms. The presence of vast numbers of cysts on the gill-lamellar tissue, up to 250 cysts per mm^2, could well inhibit adequate water passage across the gill-lamellae thereby eventually causing an anoxemia and CO_2 retention, which would ultimately lead to terminal acidosis. In conjunction with the physical inhibition of oxygenation, another closely related pathological event was observed by Ellis (1937) in his studies on heavy metal mucous precipitation and its effect on respiration. Ellis found that if the interlamellar spaces were clogged with precipitated mucus, the movement of the gill filaments became greatly inhibited or impossible. This condition had a direct effect on the blood in the gills in that blood stasis in the gill capillaries rapidly followed cessation of movement in the gill filaments. The parasitic cysts of <u>Oodinium</u> could well provide the mechanical interference necessary to inhibit the movement of the gill filaments. The rhizoids of the adult cyst could, upon penetration into the lamellar tissues, enter the delicate capillary system and act as a foci for thrombus formation and blood stasis.

CHAPTER IV

TREATMENT

Copper sulphate has been widely used in treating oodiniosis for many years. The major disadvantages of copper sulphate are its extreme toxicity to fish in soft waters and its toxic action on invertebrates. In the present investigations several drugs commonly used in the treatment of external protozoan parasites were tested for their ability to cure oodiniosis. With the exception of formalin, which is toxic at low concentrations, the other agents were selected because of their relatively nontoxic nature. Helms (1967) demonstrated that formalin reacts with dissolved oxygen thus decreasing the amount of oxygen available to fish in respiratory distress prior to the initiation of treatment. Copper sulphate has not been tested since Dempster (1955, 1956) demonstrated its curative value when the copper concentration was maintained at 0.2-0.5 ppm over a 10 day period.

Dempster (1955) showed that the parasitic cysts are rapidly detached from the epidermal surface after copper sulphate treatment. After detachment, the cysts settle to the substrate and undergo normal mitotic divisions to the 256 cell stage. Copper appears to exert its lethal action during the free-swimming dinoflagellate stage. Riley (1939) postulated that copper caused a general stimulation of movement which appears to be caused by irritation. The susceptability to copper poisoning according to Alexandrov (1935) is a function of the permeability of the integument. During the palmella stages Oodinium might be impermeable and therefore nonsusceptable to the effect of copper ions.

With the exception of the copper-tris-formalin preparation each agent was tested as a dip of 4 and 10 minutes, a bath of one hour, and a prolonged bath treatment of 12 days. The copper-tris-formalin preparation was tested only as a permanent bath agent.

Dips

Materials and Methods

Dips of formalin, malachite green, and methylene blue were tested. The following concentrations were used:

1. Formalin: 1000 ppm, and 700 ppm solutions of commercial formalin.
2. Malachite green: 2.0 ppm, 1.0 ppm and 0.5 ppm.
3. Methylene blue: 200 ppm, 100 ppm, and 50 ppm.

Five gobies with moderate infestations were placed in a one liter of test solution for 4 and 10 minutes. After removal from the test solution, the fish were placed in one gallon glass jugs with moderate aeration and observed over a 12 day period.

Results

The results of the three drugs employed in both the 4 minute and 10 minute treatments were negative. Formalin caused immediate stress followed by fatal drug toxicity within 45 minutes. Both malachite green and methylene blue at all concentrations tested failed to effect a cure with total mortality due to oodiniosis occurring in all test tanks within 5 days.

Baths

Materials and Methods

Baths of formalin, malachite green, and methylene blue were tested for one hour. Five test gobies with a known infestation were placed in a one liter beaker of test solution then after one hour were removed and placed in one gallon jugs with moderate aeration and observed over a 12 day period. The following concentrations of each test agent were employed:

1. Formalin: 1300 ppm, 1000 ppm, 500 ppm, 300 ppm, 100 ppm, and 50 ppm solutions.
2. Malachite green: 1.0 ppm, 0.5 ppm and 0.1 ppm solutions.

3. Methylene blue: 100 ppm, 50 ppm, 10 ppm, and 5 ppm solutions.

Results

Table 2 summarizes the results of the three drugs employed in the bath type of chemotherapy. In all cases the results were negative, either failing to cure oodiniosis or causing fatal drug toxicity.

The formalin treatments at higher concentrations (1300 ppm and 1000 ppm) caused a rapid stress syndrome and death within 45 minutes. At the intermediate concentrations (500 ppm and 300 ppm) formalin was only partially successful in that it caused a temporary decrease in the number of cysts; however, at the termination of the tests, many small cysts were still present on the gill and fin tissues. The lower concentrations (100 ppm and 50 ppm) were ineffectual with total mortality due to oodiniosis occurring within 72 hours.

Malachite green, at a fairly high concentration (1 ppm), had a transitory palliative action greatly reducing the cyst count for several days; however, at this concentration the fish became quite aggitated after 30 minutes. At the lower concentrations (0.5 ppm and 0.1 ppm) malachite green proved to have little or no therapeutic value. The

Table 2

Results of bath treatments of 3 chemotherapeutic agents applied for 1 hour on Quietula guaymasiae infested with Oodinium ocellatum.

Drug	Concentration (ppm)	No. of fish	Percent survival after 12 days	Remarks
Formalin	1300	5	0	fatal drug
	1000	5	0	toxicity
	500	5	100	severely infested
	300	5	100	when sacrificed at 12 days
	100	5	0	fatal
	50	5	0	oodiniosis
Malachite green	1.0	5	100	severely infested
	0.5	5	100	when sacrificed at
	0.1	5	100	12 days
Methylene blue	100	5	100	severely infested
	50	5	100	when sacrificed at
	10	5	100	12 days
	5	5	100	
Control	---	20	0	fatal oodiniosis between 8 and 12 days

fish survived the test period but were found to be heavily infested at the time they were sacrificed.

Methylene blue proved to be of questionable value. There was a 100% survival rate; however, all the fish showed moderate infestations at the time of sacrifice.

Permanent Bath Treatments

Materials and Methods

Permanent bath treatments of formalin, malachite green, methylene blue, and a copper-tris-formalin preparation were tested. The latter preparation has been used with some success by Dr. Morris H. Baslow at the New York Aquarium. The formula was given to Dr. D. A. Thomson by Dr. Baslow in a personal communication. In each case 5 to 10 fish were placed in a one gallon jug with moderate aeration. The fish were observed over a period of 12 days. During the test period all food was withheld to minimize fouling in the tanks. The following concentrations of each test agent were employed:

1. Formalin: 300 ppm and 100 ppm solutions.
2. Malachite green: 1.0 ppm, 0.1 ppm, 0.05 ppm and 0.01 ppm solutions.
3. Methylene blue: 500 ppm, 300 ppm, 200 ppm, 4.0 ppm, 2.5 ppm, and 1.0 ppm solutions.

4. Copper-tris-formalin solution: 0.15 ppm and 0.5-0.7 ppm solutions. This solution was prepared by mixing 4.0 grams copper acetate with 50 ml reagent grade formalin. To this aqueous solution 23 grams of Trizma base (tydroxymethyl amino-methate) and 23 grams of Trizma-HCL (tris hydroxymethyl aminomethane hydrochloride) was added and mixed until most of the solids had dissolved.

Results

The results of four drugs commonly employed in the permanent bath type of chemotherapy of piscine parasitosis are summarized in Table 3.

Formalin at the lower concentration (100 ppm) showed a transitory palliative action greatly reducing the cyst count for several days after which the count increased rapidly until at 120 hours post treatment, heavy cyst counts were observed. During the early phases of treatment many dead parasites were found on the substrate. At the higher concentrations (300 ppm) skin anomalies became prominent. The epidermal surface and/or congealed mucus sloughed off and there was mild exophthalmia. All the experimental fish died within 72 hours.

Table 3

Results of permanent bath treatments of 4 chemotherapeutic agents applied for 12 days on Quietula guaymasiae infested with Oodinium ocellatum.

Drug	Concentration (ppm)	No. of fish	Percent survival after 12 days	Remarks
Formalin	300	5	20	Fatal drug toxicity
	100	5	100	Severe oodiniosis
Malachite	1.0	5	60	Severe oodiniosis
green	0.1	5	60	present on all fish
	0.05	5	20	at time of death or
	0.01	5	20	when sacrificed
Methylene	500	5	100	Severe infestation
blue	300	5	100	when sacrificed
	200	5	100	at 12 days
	4	5	0	
	2.5	5	0	
	1.0	5	0	

Table 3 (continued)

Drug	Concentration (ppm)	No. of fish	Percent survival after 12 days	Remarks
Copper-tris-formalin	0.15	10	80	Few cysts present when sacrificed at 12 days
	0.5-0.7	10	100	No cysts present at 12 days
Control	---	20	0	Fatal oodiniosis between 8 and 12 days

Malachite green was found to be ineffectual at all concentrations employed. There was a transitory palliative effect in each case, i.e., a decrease in the cyst count, which was directly proportional to the concentration of malachite green employed. However, in each case at the end of 168 hours small cysts became quite numerous on the gill and fin tissues.

Methylene blue was ineffectual at all concentrations employed. The cyst count was temporarily reduced, but after 48 hours the rate of infestation increased until the fish were sacrificed, at which time many small cysts were found to be present on all fish in all test tanks. Division stages were common in the bottom detritus throughout the course of the experiment. In almost every case the division stages were heavily stained especially in the central or nuclear area. Other protozoa and occasional nematodes also took up relatively large amounts of stain. Bacteria were frequently encountered in examination of water samples. In the tanks with a concentration of less than 200 ppm there was a total mortality of the fish within 8 days. In tanks with concentrations over 200 ppm, many small cysts were present but mass mortality had not occurred before the fish were sacrificed at 12 days.

Both Malachite green and Methylene blue tended to combine with the suspended matter in the test tanks and precipitated forming a deeply colored floc.

Copper-tris-formalin solution was found to be effectual at very low concentrations. With a copper concentration of 0.15 ppm in an aquarium without molluscan shells, almost all cysts were killed within 312 hours. A few division stages were present in the detritus throughout the experiment. The greatest number of division stages were seen between 48-96 hours. However, most of these apparently failed to achieve an infective state as a fish sacrificed at 216 hours had very few cysts (less than 1.0 mm^2).

At an initial dose of 0.1 ml (0.6 ppm Cu/liter) a 100 percent cure was obtained. Division stages were present in the detritus up to 72 hours after which none were seen. All fish were sacrificed at 312 hours and were found to be completely free of cysts.

CHAPTER V

PRECIPITATION OF COPPER IONS IN SEA WATER

In the treatment of oodiniosis with copper compounds one of the major problems has been the maintenance of copper ion concentrations within nontoxic but therapeutic limits. Copper ion tends to precipitate rapidly in the presence of excess bicarbonate ion and to adhere to organic detritus thus decreasing the therapeutic value. Dempster (1955) pointed out that copper sulphate in the presence of excess bicarbonate ion substantially reduced the concentration of ionic copper and therefore he advised the treatments be conducted in the absence of all calcareous materials.

Riley (1939) showed that organic detritus may substantially reduce the concentration of ionic copper ion. He postulated that the adhesion to organic material was the major reason for the decrease in copper ion in fresh waters; however, other authorities i.e., Hutchinson (1957) stated that the inorganic precipitation is a major factor. The present investigations suggest that copper ion is being precipitated primarily as an inorganic salt.

The investigations on the precipitation rate of various copper compounds in sea water were undertaken to determine the precipitation rate in aquaria containing a calcareous substrate as contrasted with aquaria lacking such substrate and to determine what type of copper compound had the greatest residual effect.

Materials and Methods

Preparation of copper test solutions

In each of the four simple copper salts tested, the solutions were prepared in such a manner that the stock solution had a copper ion concentration of 1000 ppm. In order to determine the exact amount of salt necessary to yield a 1000 ppm stock solution the following equation, modified from Dempster (1955), was employed:

$$x = \frac{(V)\ (P)\ (Z)}{1000} \qquad \text{where,}$$

x = weight of salt in grams

V = volume in liters

P = parts per million of copper desired

Z = number of grams of salt containing 1.0 grams copper

In every case glass redistilled water was used in order to prevent contamination with other metallic ions or additional copper ion present in commercial plumbing facilities.

The following copper containing compounds were used in making stock test solutions:

1. Copper chloride: $CuCl_2 \cdot 2H_2O$ 170.52 g/mole
2. Copper acetate: $Cu(C_2H_3O_2)_2 \cdot H_2O$ 199.65 g/mole
3. Copper nitrate: $Cu(NO_3)_2 \cdot 3H_2O$ 241.54 g/mole
4. Copper sulphate: $CuSO_4 \cdot 5H_2O$ 249.5 g/mole
5. Copper-tris-formalin solution: 4.0 grams copper acetate, 50 ml of 37% formaldehyde and 46 grams tris buffer.

Copper precipitation tests

The rate of cationic precipitation at a concentration of 1.0 ppm was tested by pipetting 4.0 ml stock solution into a 5 gallon glass aquarium containing 4.0 liters of fresh sea water. Copper sulphate was also tested at a high concentration, 13 ppm, by pipetting 52 ml of stock copper sulphate solution into 4.0 liters of sea water. Each solution was tested in aquaria containing 860 grams of a calcium carbonate sand followed by a parallel series of tests in aquaria devoid of carbonate sand. Experiments were conducted over a 72 hour period except in the case of the copper-tris-formalin solution in which case tests were conducted over a 120 hour period. Water samples were analyzed at the following intervals: 0 minutes, 30 minutes, 1,2, 3, 6, 9, 15, 21, 35, 46, 58, 72, and 120 hours.

Determination of copper concentration

In the analysis of the copper concentrations determined as ppm copper ion, two separate and distinct tests were employed. In each case a Bausch and Lomb Spectronic-20 colorimeter was used to measure the percent light transmission.

Method 1.

The first method employed was the Hach Cuprethol powder technique described in the Hach Chemical Corporation Manual on "Procedures for Water and Sewage Analysis Using the Bausch and Lomb Spectronic-20 Colorimeter."

Method 2.

The second method was the sodium diethyldithiocarbamate method described by Chow and Thompson (1952) and Dempster (1955). A 50 ml sample of sea water was placed in a 125 ml separatory funnel to which was added 5.0 ml of ammonium citrate buffer pH 9.0-9.2. This solution was mixed for 30 seconds. Exactly 1.0 ml of sodium diethyldithiocarbamate indicator was then added and mixed for 30 seconds. To this solution exactly 10 ml of carbon tetrachloride was added and shaken vigorously for three minutes. The solution was then allowed to stand for 12 minutes to attain phase separation. The denser carbon tetrachloride layer was decanted into a 0.5 inch colorimetric tube and measured at a wave length of 435 millimicrons. The percent light transmission was converted to ppm copper by reference to the calibration graph constructed by Dempster (1955).

Results

In tests involving the precipitation rates of copper salts of varying molecular weights in aquaria with and without a calcareous sand substrate, a slight increase in copper ion stability was detected with increasing molecular weight, see Figures 4 through 9. Copper chloride (170.52 g/mole) in the presence of a calcareous sand substrate, forms a precipitate from sea water more rapidly than any other agent tested. During the first 2 hour period copper chloride precipitated considerably faster than the other copper salts, after which the precipitation rate decreased markedly. Copper acetate (199.65 g/mole) and copper nitrate (241.54 g/mole) precipitated at approximately the same rate. Copper sulphate (249.5 g/mole), the heaviest simple salt tested was retained in solution slightly longer than the other non-chelating agents. Copper-tris-formalin preparation had a significantly greater residual effect than any

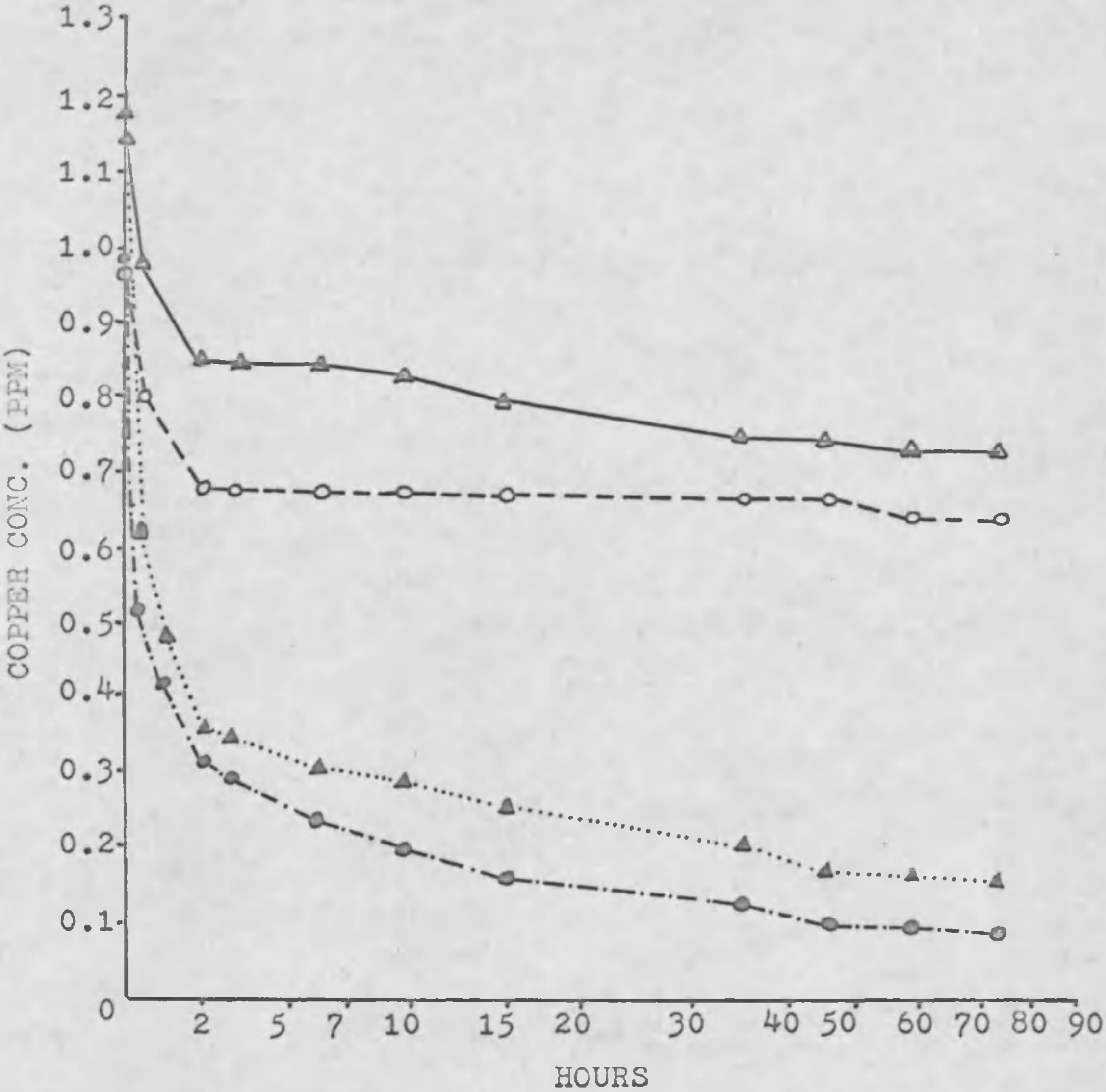

Fig. 4.--The comparative precipitation rate of a 1.0 ppm Cu^{++} solution of copper chloride in sea water aquaria containing a calcareous sand substrate and aquaria with only a glass substrate

△——△	Hach method	Glass substrate
○– – –○	Na diethyldithiocarbamate method	
▲······▲	Hach method	Calcareous sand substrate
●–·–·–●	Na diethyldithiocarbamate method	

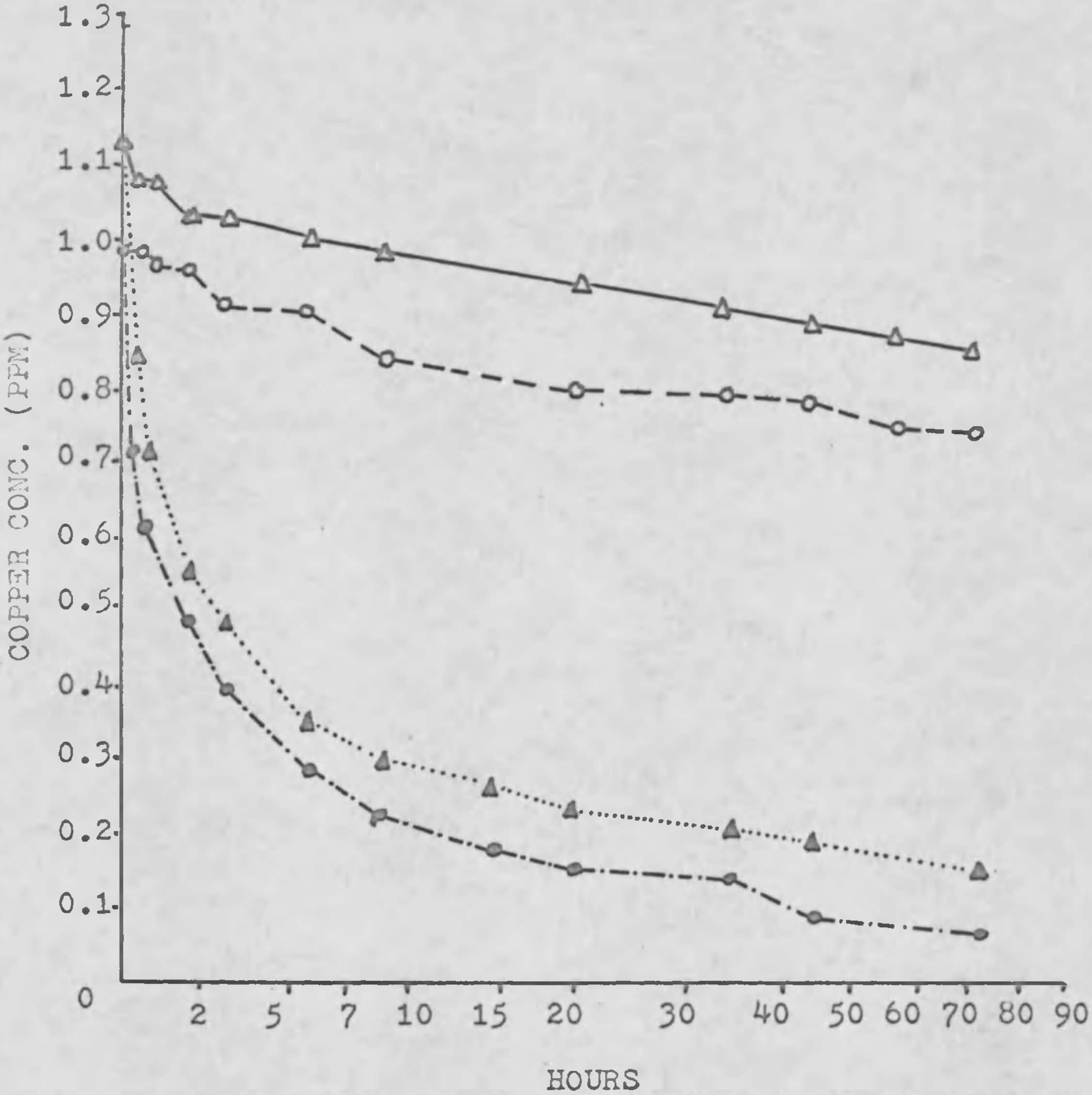

Fig. 5.--The comparative precipitation rate of a 1.0 ppm Cu^{++} solution of copper acetate in sea water aquaria containing a calcareous sand substrate and aquaria with only a glass substrate

△——△	Hach method	Glass substrate
○– – –○	Na diethyldithiocarbamate method	Glass substrate
▲·······▲	Hach method	Calcareous sand substrate
●–·–·–●	Na diethyldithiocarbamate method	Calcareous sand substrate

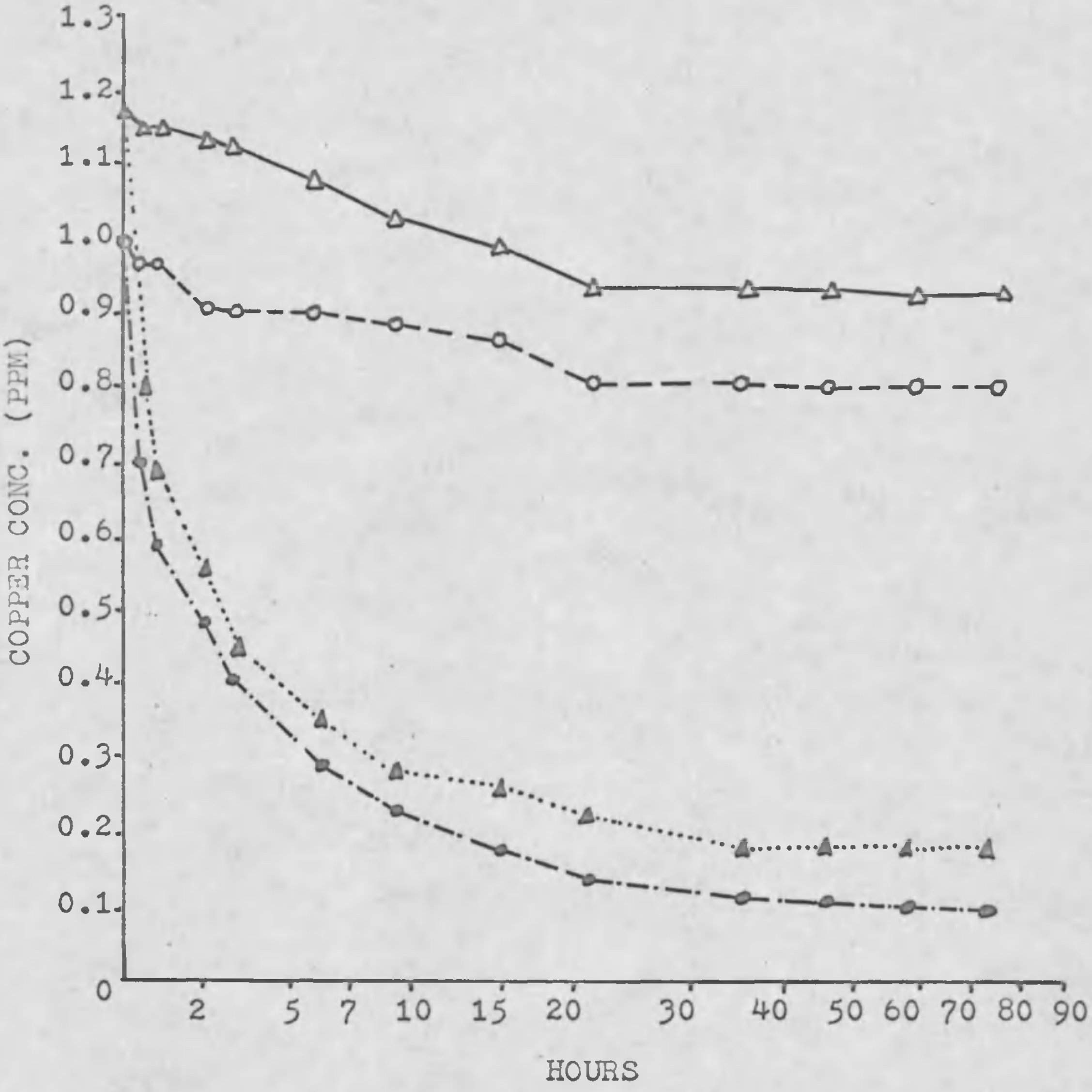

Fig. 6.--The comparative precipitation rate of a 1.0 ppm Cu^{++} solution of copper nitrate in sea water aquaria containing a calcareous sand substrate and aquaria with only a glass substrate

△———△ Hach method		Glass substrate
○– – –○ Na diethyldithiocarbamate method		
▲·········▲ Hach method		Calcareous sand substrate
●–·–·–● Na diethyldithiocarbamate method		

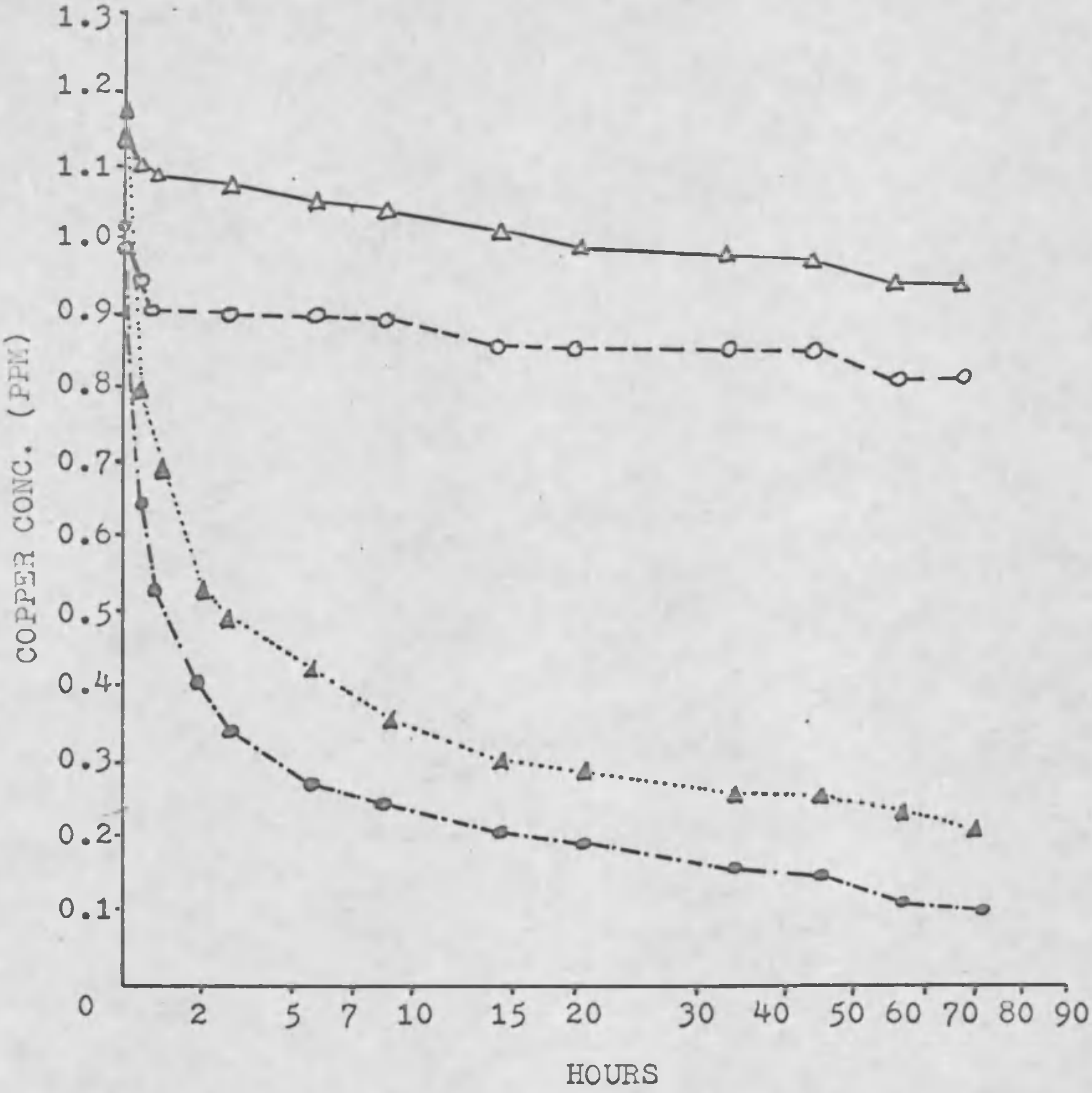

Fig. 7.--The comparative precipitation rate of a 1.0 ppm Cu^{++} solution of copper sulphate in sea water aquaria containing a calcareous sand substrate and aquaria with only a glass substrate

△——△	Hach method	Glass substrate
○---○	Na diethyldithiocarbamate method	
▲·······▲	Hach method	Calcareous sand substrate
●-·-·●	Na diethyldithiocarbamate method	

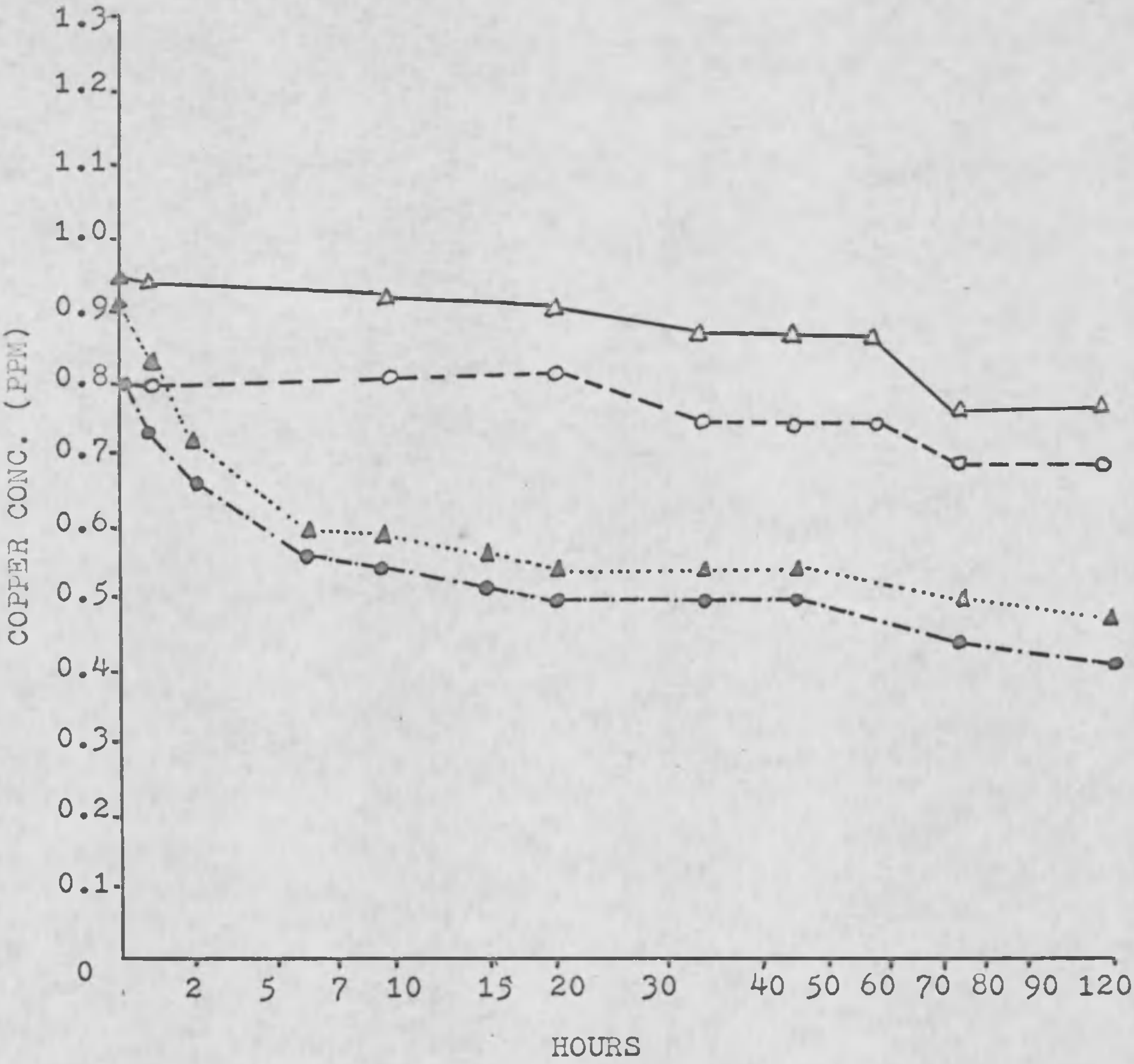

Fig. 8.--The comparative precipitation rate of a 1.0 ppm Cu^{++} solution of copper-tris-formalin solution in sea water aquaria containing a calcareous sand substrate and aquaria with only a glass substrate

△——△	Hach method	Glass substrate
○– – –○	Na diethyldithiocarbamate method	
▲·········▲	Hach method	Calcareous sand substrate
●–·–·–●	Na diethyldithiocarbamate method	

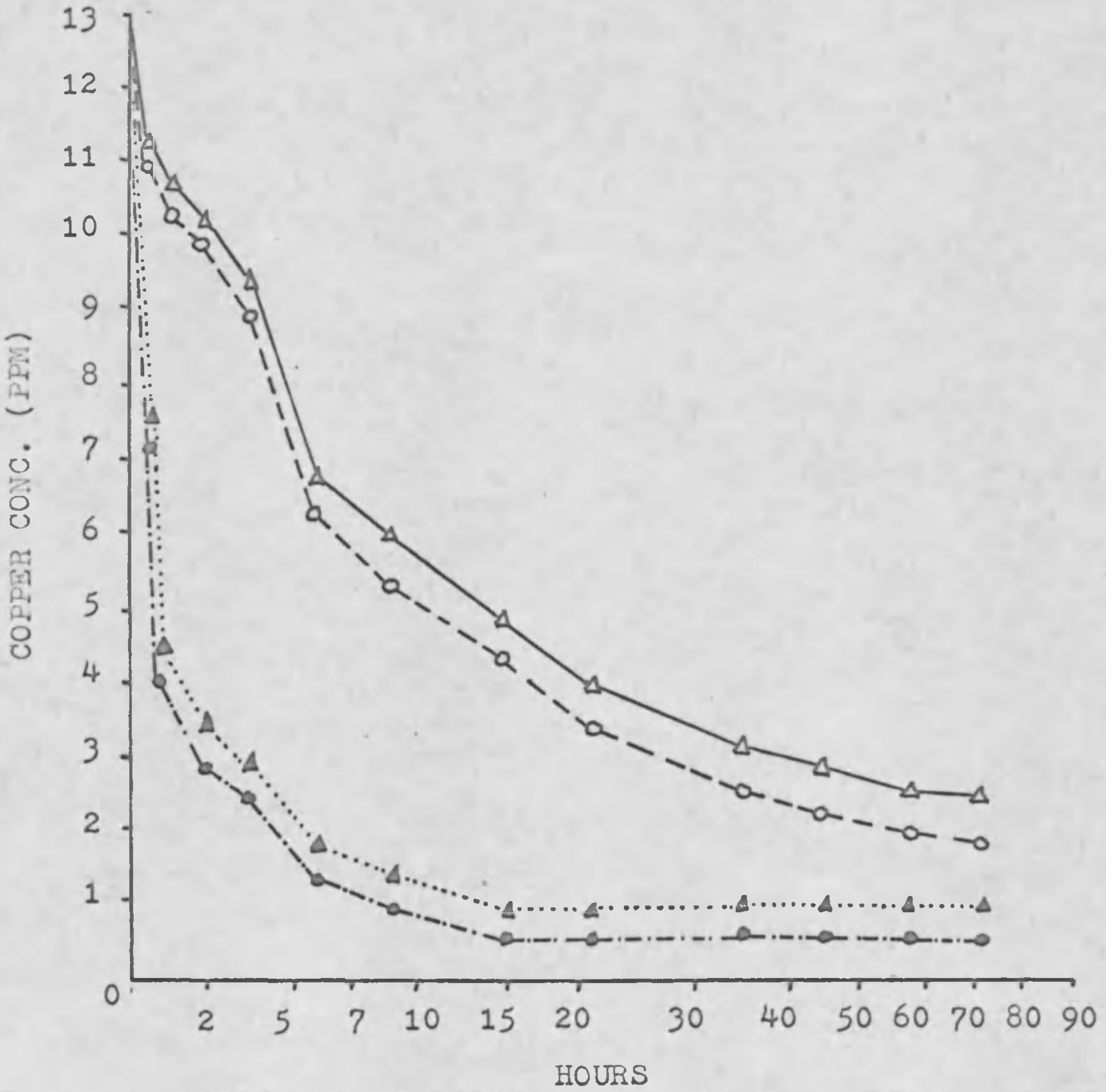

Fig. 9.--The comparative precipitation rate of a 13.0 ppm Cu^{++} solution of copper sulphate in sea water aquaria containing a calcareous sand substrate and aquaria with only a glass substrate

△——△	Hach method	Glass substrate
○— —○	Na diethyldithiocarbamate method	
▲·······▲	Hach method	Calcareous sand substrate
●—·—·—●	Na diethyldithiocarbamate method	

of the simple metallic salts. The chelated copper complex was apparently chemically bonded in such a manner that the carbonate precipitation reaction and the adsorption of copper on organic particles were partially inhibited.

The rapid removal of ionic copper from the aquaria containing a calcareous sand substrate as compared to a glass substrate is illustrated in Figures 4 through 9. Dempster (1955) pointed out that copper is rapidly precipitated from solution when excess quantities of carbon dioxide or carbonates are present. Therefore, in any treatment program it is desirable to treat the fish in clean, non-metallic tanks devoid of plants and/or calcareous materials.

Hutchinson (1957) stated that ionic copper forms a basic carbonate in hard waters. The basic copper carbonates being quite insoluble, are precipitated as a bluish-white floc which Nichols, Henkel, and McNall (1946) considered to be of somewhat variable composition. Hutchinson (1957) has postulated that these precipitations might resemble malachite $CuCO_3 \cdot Cu(OH)_2$ and azurite $2CuCO_3 \cdot Cu(OH)_2$, two well described basic copper carbonates commonly found in nature; however, the physical chemistry of the precipitates has not been verified.

CHAPTER VI

CONCLUSIONS

A parasitic dinoflagellate of the Genus *Oodinium*, resembling *Oodinium ocellatum* in every detail except being smaller, has been shown to be indigenous in the upper Gulf of California. Mild infestations were present in native gobiid fish, primarily *Quietula guaymasiae*, from the estuaries at Cholla Bay, Sonora, Mexico.

The symptoms of oodiniosis were found to be generally nonspecific, paralleling the general symptoms of anoxia and ectoparasitism by other irritating organisms. The only pathognomonic feature was a reddish mucoid secretion at the base of the pectoral and pelvic fins, which did not become evident until the infestation was well established. Diagnosis of oodiniosis from the symptoms is possible; however, final diagnosis must be based on the microscopic examination of the tissues and the demonstration of the characteristic cysts. Diagnostic examination of water samples for the free-swimming dinoflagellate stage is of questionable value as the dinoflagellate stage of *Oodinium* closely resembles the non-parasitic dinoflagellate *Oxyrrhis marina*.

Several authors, notably Jacobs (1946) and Nigrelli (1936) have reported that many fish are either moderately resistant to oodiniosis, developing only mild infestations, or after an initial infestation develop an acquired immunity. In every case *Quietula guaymasiae* developed a heavy, fatal infestation thus indicating that this species possesses little if any resistance or immunity.

Treatment using dips of formalin, malachite green, and methylene blue proved to have no therapeutic value. Formalin was toxic at the concentrations employed while malachite green and methylene blue did not inhibit the infestation.

Bath treatments employing the above mentioned drugs were only partially successful in that they effected a transitory decrease in the cyst count. It was suspected that this decrease was due to detachment of many of the cysts in the bath solution rather than actual cyst death thereby accounting for the palliative action. If detachment did occur this would account for the 100 percent survival for bath treatments of malachite green and methylene blue while considerably lower survival rates were obtained in the permanent bath treatments.

Permanent bath treatments showed varying degrees of success. Formalin treatments at a low concentration (100 ppm) failed to cure oodiniosis while a high concentration

(300 ppm) was toxic. Malachite green and methylene blue produced a transitory decrease in the cyst count; however, at the termination of the experiment all fish were heavily infested with small cysts. It was therefore concluded that malachite green and methylene blue were of no therapeutic value at the concentrations employed. The copper-tris-formalin preparation was found to be effective at very low concentrations (0.15 ppm) reducing the cyst count to less than 1 cyst per 1.0 mm^2. A fairly high concentration of 0.5-0.7 ppm resulted in a complete cure. The copper-tris-formalin preparation was not toxic to *Quietula guaymasiae* at the concentrations and times employed.

Although the copper-tris-formalin solution had a high curative effect, its value as a general chemotherapeutic agent for oodiniosis remains questionable. The Trizma base and Trizma-HCL reagents of the copper-tris-formalin preparation are considerably more expensive than reagent grade copper sulphate while being only slightly more effective. In my opinion copper sulphate applied at a concentration of 0.2-0.4 ppm as copper ion is the most economical and practical method of treating infestations of *Oodinium ocellatum*.

It was shown that the anion had a slight effect on the precipitation rate of copper salts in sea water. The precipitation rate in the presence of a calcareous sand substrate was so rapid that treatment would be difficult and would require continuous application of copper solution. The copper-tris-formalin preparation had the longest residual effect retaining a sufficient level of copper to be of therapeutic value even in the presence of a calcareous sand substrate.

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