THE LEVELS OF RESIDUAL CHLORINE IN KANEOHE BAY, OAHU, HAWAII, AND THE EFFECTS OF RESIDUAL CHLORINE ON CORAL PLANULAE

by

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INTRODUCTION

GENERAL

Coral reefs are an important feature of tropical oceans, whether as a habitat for many other organisms, as a region of high productivity for the ocean, as a source of protection for an island from waves, or as an area of recreation for skin divers. At one time southern Kaneohe Bay was described as an area of magnificent coral growth (MacKay, 1915; Edmondson, 1928). Now, however, most of the corals there have died and disappeared (Banner and Bailey, 1970; Johannes, personal communication). In addition, coral heads introduced to various stations in that section of the bay die (James Maragos, personal communication). The suspected cause of this mortality is pollution associated with the increased population growth and urbanization of the town of Kaneohe (Banner and Bailey, 1970).

EFFECTS OF VARIOUS POLLUTANTS ON CORALS

Several types of pollution may contribute to the death of corals, such as sedimentation, increased nutrient levels, alterations of salinity, changes in temperature, pesticides, oil, sewage, or chlorine in sewage. Any of these could conceivably affect either adult corals, their larvae, or their food.

A number of processes may add to sedimentation (Banner and Bailey, 1970), such as dredging (Brock et al., 1966), terrestrial erosion, reef erosion, or the addition of organic materials as in sewage. Sedimentation has increased in Kaneohe Bay in recent years (Fan and Burnett, 1969), and several investigators have shown that it affects adult corals (Mayer, 1918a; Vaughan, 1919; Mayor, 1924b; Edmondson, 1928; Marshall and Orr, 1931; Fairbridge and Teichert, 1948; van Eepoel and Grigg, 1970). Silt has also been shown to prevent coral planulae from settling (Motoda, 1939).

The elevation of levels of nutrient elements in water, such as nitrogen and phosphorous, accompanies urbanization of nearby areas. While compounds of these elements are found in the runoff from any tropical ecosystem, detergents, fertilizers, sewage treatment plants, and cesspools form a strong contribution (Banner and Bailey, 1970). Such a buildup of nutrients has occurred in Kaneohe Bay (Tseu, 1952; Bathen, 1968; Young et al., 1969). This might affect corals in a number of indirect ways (Banner and Bailey, 1970), such as causing a phytoplankton bloom and a consequent increase in turbidity. This in turn might act on the corals as sedimentation or it might reduce the light available for zooxanthellae. A phytoplankton bloom might also result in more zooplankton, the food of corals, which could be beneficial or could be harmful in excess by interfering with the feeding process (Banner and Bailey, 1970).

Another by-product of population growth is increased freshwater runoff into the ocean that results from drainage of cultivated and denuded land. This may cause a lowering of the salinity of the seawater during a heavy rainstorm. Banner (1968) observed such a phenomenon that seemed to result in the death of many corals in Kaneohe Bay, and other investigators (Rainford, 1925; Slack-Smith, 1960; Goreau, 1964; Cooper, 1966) have reported similar situations in other areas. Laboratory studies (Mayer, 1918a; Vaughan, 1919; Edmondson, 1928, 1929) have also dealt with the effects of altered salinities on adult and larval corals.

Another type of pollution characteristic of population growth is the addition of sewage. The discharge rates of sewage into Kaneohe Bay from the Kaneohe Sewage Treatment Plant and the Kaneohe Marine Corps Air Station Sewage Treatment Plant have increased in recent years (Bathen, 1968). Sewage might adversely affect corals (Mayor, 1924a) due to such accompanying phenomena as sedimentation, eutrophication, local changes in salinity, depletion of oxygen, the accumulation of toxic compounds, etc. The 96-hour TL_m (mean tolerance limit, or the concentration necessary to kill half the test organisms) of Pocillopora damicornis planulae to Kaneohe Sewage Treatment Plant effluent has been determined to be a 10-15% dilution (George Losey, personal communication, Oahu Water Quality Study).

Although not presently a problem in Kaneohe Bay, thermal

pollution, produced by industries which use seawater for cooling purposes, may well arise if the proposed power plant for Kaneohe Bay is constructed. Edmondson (1928, 1929, 1946) and Mayer (1918a; Mayor, 1918b, 1924a) have studied the effects of altered temperatures on adult and larval corals and have found that many are living near their temperature maxima. Other investigations on the effects of increased temperatures on corals of Kaneohe Bay are now being conducted (Paul Jokiel, personal communication).

Certain toxic compounds, such as pesticides carried to the ocean by streams, oil spilled into the ocean by industrial plants or tankers, or chlorine added to sewage effluents discharged into the ocean or added to seawater used in industrial cooling systems might also affect corals (Lewis, 1971). While the effect of chlorine on adult and larval corals is not known, chlorine has been shown to have adverse effects on many aquatic organisms. These will be discussed in a later section of this paper.

USES OF CHLORINATION IN WATER

Chlorine is used as a disinfectant to kill bacteria and viruses. Its best-known use has been in the purification of drinking water which was first begun on an emergency basis during epidemics in 1850 and has been used on a continuous basis since 1904 (Sawyer, 1960). Generally, in water purification a minimum total chlorine residual of 0.2-1.0 mg/l is

desirable, most of it in the form of chloramines, as the water leaves the plant and enters the distribution system (Behrman, 1968). Chlorine is also used as a disinfectant in swimming pools where free chlorine residuals should be maintained at the level of 0.4 mg/l (American Public Health Association recommendation, in Fair et al., 1968).

Chlorine has more recently been applied, somewhat less effectively, in sewage treatment plants, particularly in those which discharge into recreational areas (Scott and Van Kleeck, 1934; Rhines, 1965; Burns and Sproul, 1967). In this process, usually liquid or gaseous chlorine is introduced into the effluent at the end of primary or secondary treatment leaving a chlorine residual of approximately 0.5 mg/l as the sewage enters the receiving water (Reginald F. Young, personal communication). The Kaneohe Sewage Treatment Plant employs such a system as part of the secondary treatment, and the chlorine residual in the waste as it leaves the contact basin has been measured as 1.1 mg/l (Young and Chan, 1970). Some large cities, however, such as New York, Chicago, and New Orleans, are now switching to sodium hypochlorite (NaOCl) to eliminate the danger in handling chlorine gas and to decrease the cost of equipment (Steffensen and Hash, 1967; Baker, 1969).

Chlorination is also widely employed by power plants and other industries to discourage the accumulation of slime and the attachment of fouling organisms such as mussels in their cooling circuits (Powell, 1933; Estes, 1938; Martin, 1938;

Dobson, 1946; Turner et al., 1948). Chlorine is generally added to the water at the intake to produce a residual of 2-5 mg/l, sometimes 10 mg/l (Waugh, 1964), and the water may be in the system for about 15 minutes (Hamilton et al., 1970). The effluent from seawater-cooled power plants is often used in fish-farming experiments, and accumulation of chlorine in this water is undesirable (Page-Jones, 1971).

Chlorine is also used in the shellfish industry to disinfect the organisms before they are harvested for food. The animals are placed in tanks with water disinfected with chlorine. When the chlorine concentration drops to tolerable levels, the molluscs take it up and thus are cleansed of any organisms they may have ingested (Fair et al., 1968).

In all the uses of chlorination mentioned above, it is generally considered desirable to retain a chlorine residual at the end of treatment since the water is apt to have post-treatment contact with more bacteria, etc.

CHEMISTRY AND ACTION OF CHLORINE

The characteristic reactions of chlorine in water are due to the fact that it is a very powerful oxidizing agent. Either gaseous or liquid chlorine reacts with water itself to form hypochlorous acid and hydrochloric acid according to the equation (Sawyer, 1960; Behrman, 1968):

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^-.$$

In dilute solution and at a pH above 4, the equilibrium is strongly biased to the right-hand side of the reaction, and little Cl_2 remains in solution. Hypochlorous acid is a weak acid ($K = 2.8 \times 10^{-8}$) which dissociates to hydrogen ions and hypochlorite ions according to the equation:

$$HOCl \rightleftharpoons H^+ + OCl^-,$$

and the amounts of OCl^- and HOCl depend upon the pH. At pH 7.5, $[OCl^-] = [HOCl]$, although chloride ions affect the equilibrium. As can be seen above, the use of Cl_2 will decrease the pH, while the use of OCl^- will increase it although these effects are generally neutralized by the buffering capacity of the surrounding water. Chlorine gas, hypochlorous acid, and hypochlorite ion are often referred to as free chlorine residuals (Sawyer, 1960).

Chlorine also reacts with ammonia to form chloramines, often referred to as combined chlorine residuals. The reactions are as follows (Sawyer, 1960; Behrman, 1968):

$NH_3 + HOCl = NH_2Cl + H_2O$	monochloramine
$NH_3 + 2\ HOCl = NHCl_2 + 2\ H_2O$	dichloramine
$NH_3 + 3\ HOCl = NCl_{3(g)} + 3\ H_2O$	trichloramine

The formation of any specific chloramine compound depends upon several factors, the most important of which are pH and the relative amounts of reactants. At pH 8.3 the reaction is most rapid; above pH 4.5 dichloramine is formed, and above pH 8.5

monochloramine is formed (Behrman, 1968). When all the ammonia is converted to trichloramine or is oxidized to other gases such as nitrogen or nitrous oxide, it is called "breakpoint chlorination." Theoretically, this should require a ratio of three moles of chlorine gas to one mole of ammonia, but in practice only two moles of chlorine gas are needed. Thus, some unknown reactions are also occurring. When chlorine is added beyond the breakpoint, it remains as a free chlorine residual (Sawyer, 1960).

Chlorine also reacts with many reducing agents such as H_2S, Fe^{++}, Mn^{++}, NO_2^-, or organic compounds with unsaturated linkages (Sawyer, 1960). The "chlorine demand" of water, that is, the difference between the amount of chlorine added and the amount remaining after a certain time period, results from these reactions with inorganic and organic materials. Holland, et al. (1960) measured the immediate chlorine demand of pure seawater as 0.2 mg/l and the 30-minute demand as 4.9 mg/l. When, and only when, this demand is satisfied, an increase in chlorine dosage results in a proportional increase in free or combined chlorine residuals (Behrman, 1968).

In water purification, ammonia is generally added at the end of the treatment to convert the free chlorine to combined chlorine residuals (Behrman, 1968). If desired, dechlorination can be effected with activated carbon, but chlorine generally dissipates with time, especially in sunlight and air (James, 1941).

The bleaching effect of chlorine results from its action as a potent oxidizing agent; it literally "burns up" many kinds of organic matter, including those responsible for colors, tastes, and odors (Behrman, 1968). Both chlorine gas and hypochlorite have this same effect (Baker, 1969). Monochloramine and dichloramine also have disinfecting power, and although they are more persistent, they are not as strong a disinfectant (Behrman, 1968). The bactericidal effects of available chlorine residuals were once believed to result from simple oxidation, but it is now suspected that interference with vital enzymes is involved (Green and Stumpf, 1946). This seems especially plausible since some bacteria are more tolerant than others to the effects of chlorine (Muller, 1967). The amount of kill is proportional to the concentration of chlorine and the duration of contact (Sawyer, 1960; Burns and Sproul, 1967; Kott and Ben-Ari, 1967). Less important factors which increase the killing power are low pH (Hays et al., 1963) and high temperature (Behrman, 1968).

EFFECTS OF CHLORINE ON AQUATIC ORGANISMS

Chlorine affects many other organisms in addition to bacteria as is well-known by fish hobbyists. A number of laboratory tests have been performed to determine the tolerance limits of various freshwater organisms to chlorine. Arthur and Eaton (in press) tested the lethal and sublethal effects of chloramines on three-month-old fathead minnows (Pimephales

promelas) using a continuous-flow system with various dilutions of the chlorinated city water supply. In a 72-hour period they found 100% mortality at 154 µg/l. The TL_m for this period was between 85 and 154 µg/l. Organisms exposed for 21 weeks showed a sublethal effect of reduction in egg production. This first occurred at 16.5 µg/l, and above 85 µg/l almost no spawning occurred. Arthur and Eaton also followed the survival and growth of the larvae from the eggs of the experimental fish for the following 30 days and found impairment only at 108 µg/l. This is in agreement with the findings of Zillich (personal communication in Arthur and Eaton, in press), who, using continuous flow of chlorinated sewage effluents, found partial kills of fathead minnows in 4 days with 50-190 µg/l total residual chlorine.

Similar studies of the effects of chlorinated effluents on fathead minnows and trout have also been performed by Mr. Robert Basch of the Michigan Department of Natural Resources (Arthur, personal communication). Merkens (1958) found that about half of the experimental trout were killed in a 7-day exposure to less than 0.1 to 1 mg/l residual chlorine. While he found that free chlorine was more toxic to fish than chloramines, other researchers have found the opposite (Doudoroff and Katz, 1950; Holland et al., 1960; McKee and Wolf, 1963). The effects of chlorine on sunfish, bullheads, and trout have been studied by Coventry et al. (1935). The resistance of eels to chlorine was studied by Visintin and Errera (1958,

in Biological Abstracts, 1959). Tsai (1968) found that chlorinated sewage in the field reduced fish species diversity and abundance greatly near a sewer outfall, but he did not study the effects of chlorine alone.

Fishes are not the only freshwater vertebrates whose tolerance levels to chlorine have been determined. Kaplan (1962) found that a 10-day exposure to concentrations of 8 mg/l or greater of chlorine in a hypochlorite solution was lethal to all adult leopard frogs (Rana pipiens) tested. Sublethal effects first appeared at 4 mg/l and increased with increasing concentrations. These included subcutaneous hemorrhages, ulceration of the skin, and effects on the level of activity, muscle tonus, posture, heartbeat, and respiratory rate. Reversibility of these signs occurred only below 5 mg/l. Gill (1970) observed that brown tree frogs (Hyla ewingi) no longer reproduced in ponds when chlorination of the town water supply was initiated although adults still frequented them. In contrast, Panikkar (1960) found bullfrog tadpoles to be fairly resistant to chlorine.

Little work has been done on the effects of chlorine on freshwater invertebrates. Arthur and Eaton (in press) used their fathead minnow test method with freshwater amphipods (Gammarus pseudolimnaeus). With chloramines the 96-hour TL_m was 220 µg/l. Over a 15-week exposure a reduction of young occurred, first at 3.4 µg/l and completely at 35 µg/l. Another freshwater invertebrate, Daphnia, has been the subject

of preliminary experiments (Biesinger, personal communication in Arthur and Eaton, in press) indicating a total kill in a 3-5-day exposure to 1 µg/l chloramines.

The effects of chlorine on marine organisms have been studied less than on freshwater organisms. Both marine plants and animals have been found to be affected, however. Laboratory studies with phytoplankton (Hirayama and Hirano, 1970b) showed that a suspension of Skeletonema costatum failed to regain cell multiplication 30 days after a 5- to 10-minute exposure to 1.5-2.3 mg/l chlorine. Hirayama and Hirano concluded that the organisms were killed by the treatment. However, while Chlamydomonas sp. suffered a time lag in growth after a 5- to 10-minute exposure to chlorine, it always recovered within 9 days even in concentrations up to 20 mg/l. Hamilton, et al. (1970) found that the photosynthetic rate of a volume of water was reduced 91% during the 15 minutes it passed through the chlorinated cooling circuits of an electric plant. Bacterial densities and concentrations of chlorophyll a also decreased. While unchlorinated intake and effluent samples incubated at the temperature of the effluent had higher rates of photosynthesis than those at ambient temperature, any such temperature effect was overshadowed by the effects of chlorine in the chlorinated effluent samples. Considering the magnitude of this reduction in photosynthesis, the amount of time the plant chlorinated, and the amount of water in the estuary, they estimated a maximum loss of 6.6%

in the primary production of the estuary.

Preliminary experiments with algae (Schreiber, 1970, unpublished) showed that a 60-minute exposure to hypochlorite solution with 15 mg/l residual chlorine resulted in reductions in photosynthetic rate of 80% in Macrocystis pyrifera (brown alga), 70% in Ulva lobata (green alga), and 40% in Prionitis lanceolata (red alga). However, a drop in salinity of about 9% upon addition of the hypochlorite solution to the vessels occurred and might account for some of the reduction in photosynthesis. Nevertheless, these effects probably occurred at less than 15 mg/l because the chlorine demand of the plants incubated in a small volume of water (5 ml.) would reduce the chlorine concentration over the hour. As a part of this same project, field studies revealed that the most resistant species in the lab, P. lanceolata, was also the dominant algal species near the heavily chlorinated sewer outfalls of Pacific Grove and Carmel, California.

Respiratory rate of algae is also affected by chlorine. Yamada (1960, in Hirayama and Hirano, 1970b), found that oxygen consumption of seaweeds was increased at concentrations of chlorine less than 20-50 mg/l and decreased at higher concentrations.

Studies of the effects of chlorine on marine animals have also been conducted, primarily on those commercially important as fouling organisms or as food organisms. One organism notorious for fouling cooling circuits of industrial

plants is the mussel (Dobson, 1946; Turner et al., 1948). With a 10-minute exposure, Hauo and Chian (1958, in Waugh, 1964) determined that a chlorine concentration of 380 mg/l was required to detach adult mussels. With continuous treatment, Turner, et al. (1948) found that 10 mg/l caused a partial kill of mussels in 5 days and a total kill in 15 days. However, both Turner, et al. (1948) and Dobson (1946) suggested that low levels of chlorine might affect the larvae. This was finally tested in the laboratory by Hirayama and Hirano (1970a). Early larval stages of 2-4 cells were shown to have a TL_m of 1.1-1.2 mg/l for a 5- to 10-minute exposure. At the older trochophore stage, however, while the 10-minute TL_m was in this same range, a concentration of 2.4 mg/l was required to kill half the organisms in 5 minutes. This suggests that tolerance to chlorine increases with age. In spite of the sensitivity of the larvae, however, mussels do appear in a chlorinated system. Although only a few may succeed in attaching, those that do might protect themselves from intermittent chlorination by closing their shells (Turner et al., 1948). In addition, older mussels could take up chlorine from the water and lower the residual to which the young mussels are exposed (Waugh, 1964).

Another common marine fouling organism is the barnacle. Its naupliar larvae are also quite sensitive to chlorine. A 10-minute exposure to a chlorine concentration greater than 0.5 mg/l resulted in many deaths and a reduction in growth

rate (Waugh, 1964). Concentrations greater than 2-3 mg/l for the same time period caused almost all the larvae to die, and the few remaining survivors failed to grow. Waugh suggests that the older, pre-settlement cyprid larvae might be more resistant because of their bivalve shell, and this could explain how barnacles still settle in cooling pipes. Field observations (Holstrom, 1970, unpublished) also reveal that the toxic element of sewage on adult and larval barnacles appears to be chlorine. Holstrom found that reproduction and larval recruitment were much more drastically reduced around a heavily chlorinated outfall than a less chlorinated one.

Studies of the effects of chlorine have also been prompted on a commercially valuable marine organism, the oyster, Its larvae are extremely resistant to chlorine (Waugh, 1964). Those exposed to 10 mg/l for 10 minutes could survive and grow normally to the pre-settlement stage at which point observations were ceased. Many could grow at 50-200 mg/l. The experimental animals often did better than the controls probably because bacterial levels were lowered by the chlorine. As a result, Walne (personal communication in Waugh, 1964) now routinely exposes the oyster larvae he cultures to 3.0 mg/l of chlorine for 5 minutes to kill the bacteria on their shells. It is probably this shell that protects the oyster larvae (Waugh, 1964). Waugh also observed that Crepidula larvae and Littorina larvae, which both have a larval shell and operculum, were more resistant to chlorine than barnacle nauplii, copepods,

and other crustacean and worm larvae.

Other studies on the effects of chlorine on invertebrates include three preliminary investigations with echinoderm larvae (Muchmore, 1970, unpublished; Rotkis, 1970, unpublished; Ott, 1970, unpublished). Muchmore found that while unchlorinated sewage is a mild fertilization inhibitor, chlorinated sewage reduces fertilization rather drastically. The effect was primarily on the sperm. Sodium hypochlorite solutions also produced similar results. However, failure to use proper controls and to restore the salinity of the sewage to that of seawater make the interpretation of the data difficult.

Finally, two studies have investigated the effects of chlorine on marine fish. Holland, et al. (1960), the only researchers to use a flowing seawater system with a hypochlorite solution introduced at the intake, found toxic effects on yearling chinook salmon first appearing at concentrations of chlorine of 0.25 mg/l. A total kill occurred within less than an hour with 1 mg/l of chlorine. Another study on fish deals with the effects of low levels of chlorine on the eggs and larvae of plaice, Pleuronectes platessa (Alderson, 1970).

OBJECTIVES OF THE PRESENT STUDY

The purpose of this study was to determine the level of chlorine present in the field and the effects of chlorine on coral planulae. The field studies consisted of measuring the chlorine concentration of seawater taken from the vicinity

of two sewer outfalls in southern Kaneohe Bay, Oahu, Hawaii. The laboratory studies involved determining the lethal and sublethal effects of different concentrations of hypochlorite solutions for various time periods on the planulae.

Three techniques of assaying chlorine were used in this study, the orthotolidine test, the orthotolidine-arsenite test, and the iodometric test (Standard Methods, 1971; see Appendices A, B, and C, respectively). The first two are colorimetric methods. If residual chlorine is present in a sample, a characteristic yellow holoquinone color is produced when the sample is added to the orthotolidine reagent, and the amount of color measured corresponds to a particular concentration of chlorine. The orthotolidine-arsenite test is a modification which permits correction for the presence of other oxidizing agents besides chlorine in the sample which may react with the orthotolidine reagent. The iodometric technique is a titration method. Free iodine is liberated by chlorine in solution and is titrated with sodium thiosulfate using starch as the end-point indicator.

Chlorine assays were made near the outfalls of the Kaneohe Sewage Treatment Plant and the Kaneohe Marine Corps Air Station Sewage Treatment Plant. The outfalls are about 600 meters and 200 meters from shore, respectively, and about 8 meters and 7 meters deep, respectively. Their locations in Kaneohe Bay are marked in Figure 1. Water samples were collected from the surface boils in a dark glass bottle with a capacity of approximately 500 ml. The day of the week and the time of the day of collection were recorded. Subsurface samples from the boils were obtained with a Van Doren water sampler.

Upon collection the samples were placed in the shade and

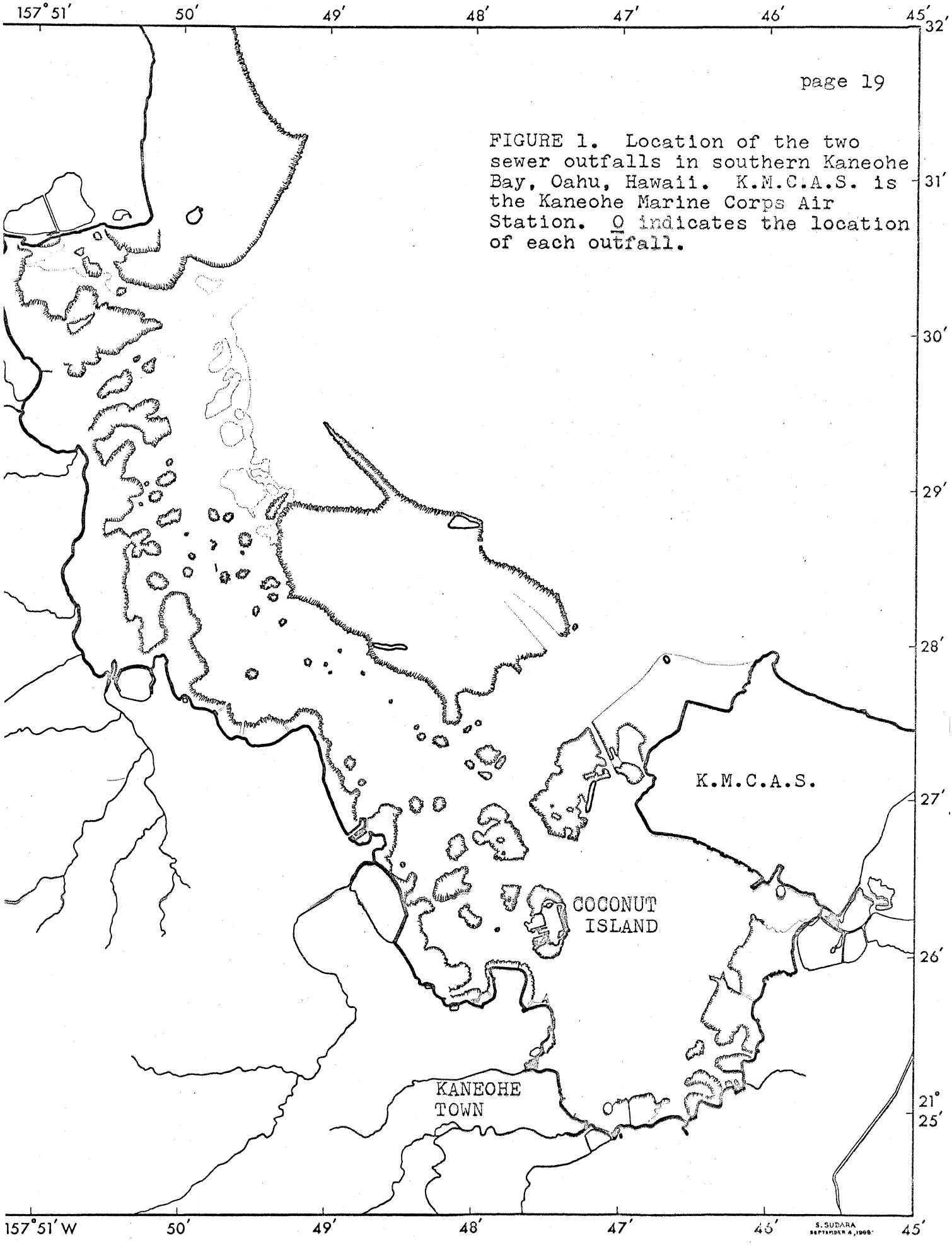


FIGURE 1. Location of the two sewer outfalls in southern Kaneohe Bay, Oahu, Hawaii. K.M.C.A.S. is the Kaneohe Marine Corps Air Station. O indicates the location of each outfall.

returned quickly to the laboratory and added to the orthotolidine reagent exactly 8 minutes later for the orthotolidine test and 10 minutes later for the orthotolidine-arsenite test. For the former technique both the sample and the reference solutions (see Appendix A) were then placed in the dark for two minutes to minimize interferences by nitrites. Exactly four minutes after addition to the reagent, the absorbance of the sample relative to the reference was read on a Beckman 109200 Model DU-2 spectrophotometer. This absorbance value was corrected for turbidity (see Appendix A), and the concentration of chlorine corresponding to the corrected absorbance value was then read from a calibration curve plotted for that day.

However, the presence of interfering substances in the sewage was suspected when two samples were retested 20-25 minutes after initially being tested, and the apparent chlorine concentration in both had not dropped. The determined chlorine residual of approximately 0.01 mg/l would not be expected to persist in seawater for that period of time (see Table 3). Therefore, the orthotolidine-arsenite technique, which does allow corrections for interferences, was used for later tests. The absorbance of the sample relative to the reference (see Appendix B) was read as above exactly four minutes later, and the corresponding chlorine concentration was calculated. After the chlorine content was determined by either method, the pH of the sample was determined with a Beckman Zeromatic (SS-3) pH meter and the salinity with an American Optical

Model 10402 T/C refractometer.

Laboratory experiments were performed to determine the tolerance levels of three species of coral planulae to chlorine in solution. Two hermatypic species, Pocillopora damicornis Linnaeus and Cyphastrea ocellina Dana, and one ahermatypic species, Tubastrea aurea Quoy and Giamard (= Dendrophyllia manni) were used. Each species of planulae was collected by a different method. In Kaneohe Bay P. damicornis planulates naturally for several days during full moon throughout the year but especially in late summer and fall (June Harrigan, personal communication). A few days before this period, several healthy coral heads were collected and placed in an aquarium supplied with constantly flowing water from the seawater system. A tube led from the overflow outlet to a container with only a screen across the bottom sitting in a finger bowl. Thus, as the planulae were released by the parent polyps, they floated out of the aquarium and were collected in the container. Planulae were collected from this species daily for each experiment from November 2 to November 5, 1971, and again on December 5, 1971.

While P. damicornis planulates only at a certain time of the month, C. ocellina planulates almost continuously (Edmondson, 1929). Edmondson's method of collecting adult corals, placing them in water in a finger bowl, and heating them to approximately 33° C. until the polyps released their planulae, was used. While he reported that planulae of this

species could be collected every month of the year, Dr. S. Arthur Reed and Dr. Austin Lamberts failed to obtain any from August to October, 1971 (personal communication). Thus, the only specimens collected for the present experiments were obtained on November 2, 1971, by this method, and these were smaller and lighter in color than are normal ones.

The third species, T. aurea, has been observed to planulate only in August and October (Edmondson, 1929). Adults of this species were placed in an aquarium in the seawater system, and on October 23, 1971, several planulae were released by each polyp. These were simply pipetted from the adult's mouth. Smaller samples from this single batch of planulae were used for each of the experiments on October 25-28, 1971. There were no visible differences among those used on the different days.

Chlorine solutions of varying concentrations were prepared by adding appropriate amounts of a 5 percent sodium hypochlorite solution (Matheson, Coleman, and Bell Company) to seawater that had been filtered three times through an Aqua Pure filter to remove particulate materials that might increase the chlorine demand. The experimental vessels for the different concentrations or exposure times were set up in a randomized fashion to avoid bias in selection of planulae with respect to age, activity, etc.

In the experiments with Tubastrea, the orthotolidine technique was used to measure the residual chlorine content

of the water. The times between the various steps of this procedure were kept constant for all experiments. One minute after the solution was mixed, the assay for the concentration of chlorine was begun. One minute later, 100 ml. of the chlorine solution were added to a 150 ml. beaker, and 10 planulae were pipetted into this from a common source for the day. All the planulae in the common source had been judged healthy by their dark color and their characteristic spiral swimming pattern in the aboral direction. Four minutes after the assay was begun, the absorbance of the sample relative to the reference (see Appendix A) was read spectrophotometrically, and the concentration of chlorine was calculated. Following exposure times ranging from 10-60 minutes, the planulae were removed to fresh filtered seawater for observation. The chlorine concentration of the incubation solution was retested to determine the amount of breakdown of the chlorine due to the chlorine demand of the seawater, any materials in the seawater, any substances in the glassware, and the planulae.

Since Tubastrea planulae were found to be resistant to chlorine concentrations up to 10 mg/l, the iodometric technique was employed in the subsequent experiments with Pocillopora to allow the use of concentrations up to 40 mg/l. As in the previous experiments, one minute after the chlorine solution was prepared, the assay was begun. The planulae were then added to the beaker containing 100 ml. of chlorine solution, and while they incubated, the titration was performed.

Unfortunately, it was impossible to end the assay within equal time intervals as was possible with the orthotolidine technique. At the end of exposure times ranging from 10-60 minutes, the planulae in all but one experiment were removed to fresh seawater for observation. In this experiment the planulae were transferred every hour for 7 hours to new chlorine solutions of approximately 5 mg/l before being transferred to fresh seawater. The chlorine solution was not reassayed at the end of the exposures for two reasons. First, previous experiments with Tubastrea showed that the concentrations of 5 mg/l and above did not break down appreciably in 60 minutes. Second, a 500 ml. sample was generally required for the titration while only 100 ml. of incubation solution were available.

After removal to fresh seawater, the planulae in all the experiments were kept in beakers in natural daylight in an air-conditioned room with a temperature of 23° C. The water in the beakers was replaced once a day initially and less often later after it was observed that it was not necessary to change it so often. The animals were examined 24 hours after exposure to chlorine for mortalities, and their development was followed for several days afterward. Planulae were known to be dead when they began to disintegrate. Initially, planulae that appeared to be abnormal were separated to individual petri dishes for further observation, but some of these then settled on the plastic whereas the other larvae in the beakers were not settling on the glass. To avoid offering different

substrates for settling to the planulae, abnormal ones were no longer isolated.

Etched slides with an algal film were introduced into the beakers in all of the experiments with Pocillopora except the 7-hour one to offer a preferred substrate for settling (June Harrigan, personal communication).

LEVELS OF CHLORINE PRESENT IN THE FIELD

The results of the chlorine assays of samples taken from the surface boils from the Kaneohe Sewage Treatment Plant outfall and the Kaneohe Marine Corps Air Station outfall are given in Tables 1 and 2, respectively. The amounts of chlorine measured with the orthotolidine-arsenite test, which corrects for interfering substances, are lower than those determined with the orthotolidine method (Table 1) although it might be argued that this is due to the fact that 8 minutes elapsed between collection and testing of samples with the orthotolidine technique whereas 10 minutes elapsed with the orthotolidine-arsenite technique. Nevertheless, with either test, the levels of chlorine measured in the Kaneohe Sewage Treatment Plant and the Kaneohe Marine Corps Air Station Sewage Treatment Plant surface boils were 0.01 mg/l or less.

The pH of the samples ranged from 8.03-8.38, the average being 8.17, and this is comparable to the average pH of seawater (pH 8.1, Sears, 1961). The salinities ranged from 34-35 o/oo in the Kaneohe Sewage Treatment Plant boil and 32-33 o/oo in the Kaneohe Marine Corps Air Station Sewage Treatment Plant boil and these values are only slightly lower than the average range of salinities measured by Bathen (1968) in Kaneohe Bay during the months of September and October (34.8-35.3 o/oo).

In spite of the fact that fresh water, being less dense

TABLE 1. Levels of chlorine detected in the surface boil of the Kaneohe Sewage Treatment Plant
(values in parentheses are extrapolated)

test	day	time	chlorine conc'n (mg/l)	pH	salinity (o/oo)	tidal height
OT[1]	Mon.	4:03 pm	0.010	-	-	1.3
OT	Tues.	10:10 am	0.010	-	-	0.6
OT	Mon.	9:33 am	0.013	8.08	-	2.0
OT	Tues.	9:19 am	0.012	8.03	-	2.0
OT	Thurs.	10:20 am	(0.001)	8.17	35	1.5
OTA[2]	Sat.	9:27 am	(0.005)	8.38	35	0.9
OTA	Sat.	12:20 pm	(0.007)	8.35	35	1.3
OTA	Sat.	3:56 pm	(0.006)	8.30	35	0.7
OTA	Sun.	9:45 am	0.010	8.25	34	0.7
OTA	Sun.	11:30 am	0.010	8.25	34	0.9
OTA	Sun.	2:50 pm	(0.006)	8.29	34	0.8

[1]orthotolidine test
[2]orthotolidine-arsenite test

TABLE 2. Levels of chlorine detected in the surface boil of the Kaneohe Marine Corps Air Station Sewage Treatment Plant
(values in parentheses are extrapolated)

test	day	time	chlorine conc'n (mg/l)	pH	salinity (o/oo)	tidal height
OTA[1]	Sat.	12:18 pm	0	8.10	33	0.2
OTA	Sat.	4:13 pm	(0.002)	-	-	0.4
OTA	Weds.	12:27 pm	(0.002)	8.08	33	0.9
OTA	Weds.	4:16 pm	(0.006)	8.05	33	0.2
OTA	Thurs.	2:14 pm	(0.002)	8.05	32	0.6
OTA	Thurs.	4:00 pm	(0.004)	8.05	32	0.0

[1]orthotolidine-arsenite test

than salt water, rises to the surface, the salinities measured in the boils were approximately equal to that of seawater. This indicates that considerable mixing of chlorinated sewage and seawater has occurred, and so a deep-water sample was considered necessary.

However, samples taken at depths of 7m and 5m near the Kaneohe Sewage Treatment Plant and the Kaneohe Marine Corps Air Station Sewage Treatment Plant boils, respectively, had salinities of 35 o/oo and 32 o/oo, respectively, and both had less than 0.01 mg/l chlorine. At the time both samples were taken, the water was quite turbulent as it usually is during the winter, and appreciable mixing had no doubt occurred. Because of their salinities, the samples cannot be regarded as representing the sewage just as it leaves the outfall pipe.

LABORATORY EXPERIMENTS WITH CORAL PLANULAE

The assayed chlorine concentrations and the exposure times used in the experiments are given in Table 3 for T. aurea and in Table 4, parts A through E, for P. damicornis. The C. ocellina larvae were exposed to the same conditions as the P. damicornis planulae in Table 4A. As can be seen in Table 3, the chlorine levels dropped with time, especially in the first 10 minutes and in the lower concentrations.

Planulae of all species were immobile and contracted throughout the exposure periods in chlorine concentrations of 0.49 mg/l or greater. In the lower concentrations the planulae

TABLE 3. Initial and final concentrations of chlorine in experiments with Tubastrea planulae (excluding controls)

experiment	exposure time (min.)	concentrations (mg/l) of chlorine before Planulae added	concentrations (mg/l) of chlorine after planulae added	percent breakdown
T-I	10	0.018	0	100
T-I	10	0.056	0.016	71.3
T-I	10	0.49	0.47	4.09
T-I	10	10.0	10.0	0
T-II	10	<0.010	0	100
T-II	10	0.060	<0.010	83.5
T-II	10	0.66	0.59	10.6
T-II	10	10.0	10.0	0
T-III	10	9.2	8.8	4.35
T-III	15	8.2	7.9	3.65
T-III	20	8.2	7.9	3.65
T-III	15	0.60	0.58	3.33
T-III	15	0.66	0.60	9.10
T-IV	60	4.1	3.6	12.2
T-IV	60	5.3	4.6	13.2
T-IV	60	6.0	5.2	13.3
T-IV	60	≥10	≥10	---

immediately "balled up," but within a few minutes they began swimming. In all the controls the T. aurea planulae began swimming actively immediately after being pipetted into unchlorinated seawater, and the other species generally did so within a short time. Recovery times in fresh seawater varied from approximately 10 minutes after a 10-minute exposure to 0.49 mg/l chlorine to approximately 70 minutes after a 20-minute exposure to 8.2 mg/l chlorine, to several hours after longer exposures to higher concentrations.

In the days following exposure, the general behavior of the experimental planulae of all 3 species did not differ from the controls. The larvae were either elongated, contracted, or some intermediate shape. They either floated, attached to the container by a thin filament, or swam aborally and/or spiraled. They sometimes flattened against the container. The T. aurea planulae tended to be the most active of the 3 species, P. damicornis the least active. The only trend noted among all the planulae was that the experimental and control animals alike tended to become less active after a few days although they could generally be induced to swim with a blast of air from a pipette. Such reversible behavior has been described as characteristic for many species of coral planulae (Edmondson, 1929; Atoda, 1947; June Harrigan, personal communication; S. Arthur Reed, personal communication).

The appearance, development, and mortality of planulae will be described separately for each species. T. aurea

planulae are dark orange in color like the adult corals, and the aboral half is often lighter than the oral half. A thin clear ectoderm with many constantly moving cilia can be seen. No differences in color were noted between the experimental and control animals.

The only distinct changes in development seen in T. aurea planulae 30 days after exposure to chlorine were tentacle formation in planulae, settlement of planulae, and mortality of either planulae or the newly-settled polyps. No abnormalities in shape, swimming pattern, or development were seen.

Very few planulae settled or died. The first to settle, a control (T-III, 0 mg/l), did so 3-4 days after the experiment was set up. Two more planulae settled 20-22 days after exposure to the highest concentration for the longest time in the experiments with T. aurea (T-IV, ≥10 mg/l, 60 min.). The only others to settle in 30 days were 3 more in the same beaker and 5 in T-IV, 6.0 mg/l, 60 min. With additional time more planulae began to settle, sometimes collectively near the water level, but no effects of chlorine on settling were seen.

The first two deaths occurred in controls, one newly-settled polyp, the first to settle (T-III, 0 mg/l), died in 8-10 days and one planula (T-I, 0 mg/l) died in 19-23 days. The only other deaths in 30 days, 5 of them, occurred 27 days after exposure to 0.66 mg/l for 15 minutes (T-III).

Tentacle formation in the free-swimming stage occurred in only 3 beakers. While 3 planulae in T-IV, 6.0 mg/l, 60

min. and 1 in T-III, 0 mg/l developed tentacles, all 10 of those exposed 15 minutes to 8.2 mg/l chlorine did. While this latter case is most interesting, it does not appear to be attributable to chlorine.

The C. ocellina larvae were brown in color due to the presence of zooxanthellae, but they were lighter in color than P. damicornis. Changes in development seen in this species up to 22 days following exposure to chlorine were larval settlement and abnormalities in larval shape. Although all of the planulae appeared to be immature due to forced expulsion from the parent, none of them died in the 22 days. Thirteen to fifteen days after a 10-minute exposure to approximately 5 mg/l and 10 mg/l chlorine, 1 and 3 planulae settled, respectively. In the next 7 days, 2 settled in the control. During the same time, 2 planulae in the control developed very odd shapes as did 1 of those that had been exposed to 5 mg/l. Short exposures to chlorine up to 15 mg/l thus appear to have no visible effects on C. ocellina planulae.

P. damicornis larvae are dark brown in color due to many zooxanthellae contained in endodermal cells in the interseptal areas. The arrangement of the zooxanthellae results in striations running the length of the body. While portions of this pattern were occasionally bleached in some animals, this did not occur preferentially among the experimental or control organisms. Like T. aurea planulae a clear ectoderm with cilia is characteristic of P. damicornis larvae.

Several events in the development of the experimental and control larvae were observed. Abnormalities were occasionally seen in shape, movement, or development of the mesenteries. In the latter instance, while normal planulae would develop twelve equal chambers separated by the mesenteries, the abnormal ones would have several normal chambers but a few especially large, indistinct, bleached ones. The numbers of mortalities or abnormalities in the days following exposure to chlorine were noted. While the numbers of deaths were cumulative, the numbers of animals with abnormalities were not since some died and others later appeared indistinguishable from normal organisms.

The introduction of settling slides no doubt created different conditions in the beakers due to differences in the degree of etching and the amount of algal film. Their addition may have fouled the water and thus increased the bacterial levels and/or depleted the oxygen content. Since the introduction of the slides weakened possible comparisons between and within experiments, each experiment was treated separately.

The Kolmogorov-Smirnov test (Tate and Clelland, 1959) was used to indicate deviations from randomness in the numbers of abnormalities or mortalities in each beaker on each day. This test involves determining the total number (N) of abnormalities and deaths each day and calculating the cumulative percent of this total occurring in each beaker. The maximum deviation (D) from an expected distribution (assuming N to be

equally distributed) indicates the probability (P) of random deviation from the expected values. The results of this test are summarized for each of the 5 experiments with P. damicornis planulae in Table 4, parts A through E. The expected and observed cumulative percents are plotted in Figure 2, parts A through E.

As can be seen in Table 4 and Figure 2, very few abnormalities and deaths occurred in all of the experiments initially. This made it more difficult to detect significant differences between the experimental and control animals. However, in all of the experiments, before the addition of the slides, there was no significant deviation from randomness. Only on the first day of observation of experiment P-I was there a hint of significance ($0.10<P<0.15$) because one abnormality occurred in each of the 4 highest concentrations. However, if the results for the first day of observation of experiments P-0, P-I, and P-II are combined (no slides had been added yet), the probability of deviation from randomness is greater than 0.20.

After the slides were added, the trend of numbers of abnormalities and deaths increasing with chlorine concentration became more pronounced in experiment P-I until it reversed on the sixth day. In experiment P-II abnormalities and deaths occurred more frequently in the larvae exposed to lower concentrations than higher ones after the slides were added, and on the seventh day this was significant ($P<0.01$). Again, this

TABLE 4. Cumulative percents of abnormalities and deaths of P. damicornis occurring on the days following exposure of planulae to chlorine solutions of various concentrations for various times (D = maximum deviation, N = number of abnormalities and deaths, P = probability of rejection; each value is for one beaker with 10 planulae unless otherwise noted)

A. Experiment P-O[1]

exposure time (min.)	10	10	10	10			
chlorine conc'n (mg/l)[2]	0	5	10	15	D	N	P
days after exposure							
1	0	50.0	50.0	100	25.0	2	>0.20
4	0	50.0	50.0	100	25.0	2	>0.20
s l i d e s a d d e d							
8	0	50.0	50.0	100	25.0	2	>0.20
10	0	100	100	100	75.0	2	>0.20
15	21.4	57.1	71.4	100	7.1	14	>0.20

[1]Only 5 planulae per beaker
[2]Chlorine concentrations not assayed

B. Experiment P-I

exposure time (min.)	10	10	10	10	10	10	
chlorine conc'n (mg/l)	0	5.53	10.2	15.0	22.9	23.0	cont. on next page
days after exposure							
1	0	0	0	0	0	25.0	
s l i d e s a d d e d							
2	0	0	0	0	0	28.6	
3	0	0	0	0	0	20.0	
6	41.7	41.7	58.3	58.3	58.3	66.7	
8	22.2	26.7	37.8	48.9	68.9	71.1	

B. (cont.)

exposure time (min.)	10	10	10			
chlorine conc'n (mg/l)	26.9	32.9	37.9	D	N	P
days after exposure						
1	50.0	75.0	100	55.5	4	0.10<P<0.15
s l i d e s a d d e d						
2	42.9	71.4	100	55.5	7	0.01<P<0.05
3	40.0	80.0	100	55.5	5	0.05<P<0.10
6	83.3	91.7	100	30.6	12	0.10<P<0.20
8	77.8	86.7	100	13.4	45	>0.20

C. Experiment P-II

exposure time (min.)	10	10	10	10	10	10			
chlorine conc'n (mg/l)	0	18.6	23.2	28.1	31.8	38.5	D	N	P
days after exposure									
1	0	0	50.0	100	100	100	33.3	2	>0.20
2	0	25.0	75.0	100	100	100	33.3	4	>0.20
s l i d e s a d d e d									
5	0	71.4	85.7	100	100	100	38.1	7	=0.20
7	7.14	78.6	85.7	92.9	100	100	45.3	14	<0.01

D. Experiment P-III

exposure time (min.) / chlorine conc'n (mg/l)	10 / 0	10 / 5.20	30[1] / 5.20	60[1] / 5.20	D	N	P
days after exposure							
slides added	100	100	100	100	75.0	1	---
1	10.5	47.4	57.9	100	17.1	19	>0.20
4	14.3	52.4	62.0	100	13.0	21	>0.20
6	8.3	45.8	62.5	100	16.7	24	>0.20
9							

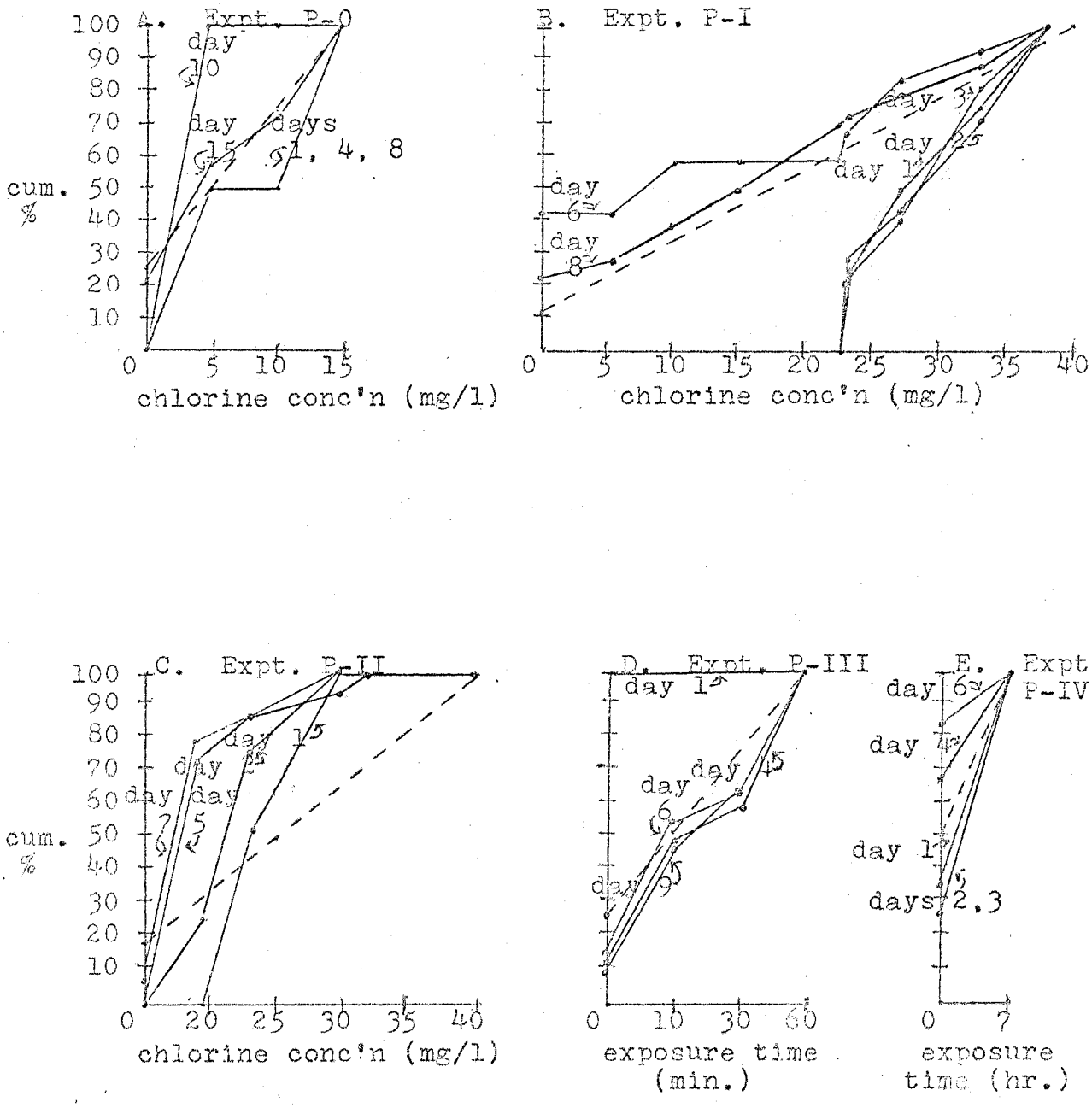
[1] Two beakers with 10 planulae each

E. Experiment P-IV[1]

exposure time (min.)	420[2]	420[2]			
chlorine conc'n (mg/l)	0	4.81±0.82	D	N	P
days after exposure					
1	33.3	100	16.7	3	>0.20
2	25.0	100	25.0	4	>0.20
3	25.0	100	25.0	4	>0.20
4	66.7	100	16.7	3	>0.20
6	83.3	100	33.3	6	>0.20

[1] No slides added
[2] Five beakers with 10 planulae each

FIGURE 2. Expected and observed cumulative percents of abnormalities and deaths of P. damicornis occurring on the days following exposure of planulae to chlorine solutions of various concentrations for various times (dotted lines represent expected values, solid lines represent observed values)



significance may be due to different conditions created by the slides. In all of the other experiments with P. damicornis, the addition of slides was accompanied by no significant differences in the numbers of abnormalities and deaths between the experimental and control organisms ($P>0.20$).

Because of the differences between slides, data analysis was not performed on the numbers of planulae settled. Nevertheless, many did settle in both experimental and control beakers, initially on the slides and later also on the container as the glass acquired a film. The basal plate, septa, and the calyx developed normally.

Tentacle formation in planulae was also common and has previously often been observed in P. damicornis (Edmondson, 1929; June Harrigan, personal communication).

On the days of observations given in the tables, only one P. damicornis polyp developed buds. One exposed 10 minutes to 15 mg/l chlorine (P-0) developed 5 buds between the 4th and the 8th days after planulation and exposure to chlorine. Many other experimental and control polyps later developed buds.

The assayed chlorine in surface and subsurface water samples in boils of the Kaneohe Sewage Treatment Plant and the Kaneohe Marine Corps Air Station Sewage Treatment Plant were 0.01 mg/l or less. However, in the 8-10-minute period between sample collection and testing, the chlorine in the seawater containing organic matter may have dropped from about 0.05 mg/l (see Table 3). Addition of the sample to the reagent immediately upon collection in the field produced no visible color. Therefore, since the permanent color standards for concentrations of 0.05 mg/l and above have visible color, the level of chlorine in the seawater must be less than 0.05 mg/l.

The total chlorine residual measured in the Kaneohe Sewage Treatment Plant effluent by the orthotolidine method is 1.1 mg/l (Young and Chan, 1970). The chlorine dosage is generally kept constant and is not regulated according to load (Reginald F. Young, personal communication). Although sewage and storm water are often chlorinated together (Camp, 1961), storm water does not pass through the sewage treatment plants on Oahu (Reginald F. Young, personal communication), and thus excessive rainfall would not alter the chlorine residual in the effluent.

The level of chlorine drops due to the dilution by the large volume of seawater and the reactions with seawater and the organic matter in it. The mixing of chlorinated sewage with the seawater is increased at the Kaneohe outfall by the fact that the outfall pipe has several holes, resulting in about

10 boils in a line. This dilution is greater in the winter than the summer because stratification of the bay is decreased by the waves caused by the strong winter tradewinds (Bathen, 1968).

Very low levels of chlorine are also to be expected in the seawater near outfalls from other sewage treatment plants on Oahu since the reported residuals in the effluents are comparable to that of the Kaneohe Sewage Treatment Plant. The levels that have been determined are (Young and Chan,, 1970):

plant	residual chlorine (mg/l)
Palisades	1.5
Kailua	0.1
Waipio	1.5
Wahiawa	0.2
Maunawili	0.7

That all of the species of planulae tested in the laboratory were immobile in solutions of 0.49 mg/l chlorine or greater is of real importance. This indicates that the planulae are sensitive to chlorine although short-term exposures up to 7 hours do not produce irreversible effects. This suggests that they may not recover from long-term exposures to chlorine. Perhaps the immobilization of the planulae might cause them to fall upon an unfavorable substrate such as mud.

The experiments performed in this study indicate that coral planulae are much more tolerant to chlorine than many marine forms previously studied. They may have a resistance comparable to that of oysters (Waugh, 1964). Perhaps this is due to the fact that coral planulae are more highly developed than many marine larvae such as the 2-4-cell stage or trochophore larva of mussels or the nauplius larva of barnacles. It is no doubt advantageous for corals to release their young to the environment in this highly developed state rather than as eggs. Planulae characteristically have two layers of cells, the outermost epidermis which includes nerve cells and elastic fibers, and the endoderm. A thin mesogleal layer lies between these two (Edmondson, 1929). While the shell of an oyster larva may provide that organism with some protection from the oxidizing action of chlorine, the epithelium of a coral planula, composed of many thick, slender columnar cells with numerous nematocysts and glands (Edmondson, 1929), might perform a similar function.

To the author's knowledge, mucous production has not been reported for larval corals as it has been for adults. However, if this does exist, the oxidizing action of chlorine would be spent more on the mucous than the surface of the planulae.

While solutions of up to 40 mg/l chlorine have no noticeable effect on coral planulae, chlorine might interact with other factors in the field and become more toxic. Either the action of chlorine itself could be modified by such factors,

or the factors could interact by lowering the tolerance of organisms to their combined effects. The toxicity of chlorine would probably not be increased by the pH or BOD (biochemical oxygen demand) of the sewage, however. The pH measured in a number of Oahu sewage treatment plant effluents ranged from 6.50-7.97 (George Losey, personal communication, Oahu Water Quality Study). In the surface boils from the two sewage treatment plants studied, the pH had increased to 8.17, and chlorine is less potent in an alkaline solution than in a more acidic one (Hays et al., 1963). Chlorine reduces BOD (Enslow, 1932; Snow, 1952; Tsai, 1968) and in that respect improves water quality. Organisms might, however, be affected by simultaneous exposure to chlorine and other constituents of sewage or the low salinity of sewage. For instance, Allen, et al. (1946, 1948) found that addition of small amounts of chlorine to sewage effluents containing thiocyanate (CNS) produced a toxic compound believed to be cyanogen chloride (CNCl). Although no chlorine residual was detectable in the effluent, the sewage reduced fish survival much more than unchlorinated sewage.

In addition, chlorine and high temperatures in power plant effluents might interact, especially since high temperatures enhance disinfection by chlorine (Behrman, 1968). Hirayama and Hirano (1970b) tested the effects of chlorine and high temperatures separately on phytoplankton, and Waugh (1964) tested the effects of these two factors combined on oyster

larvae.

However, while coral larvae are quite tolerant of chlorine in seawater in the laboratory, adult corals might be more sensitive. Edmondson (1928, 1929) tested the viability of adult corals and their larvae under various environmental conditions and found that Cyphastrea ocellina and Dendrophyllia manni (=Tubastrea aurea) planulae endured high temperatures better than adults and that C. ocellina planulae tolerated low salinities better than adults. As a part of the present study, preliminary experiments were performed with adult Fungia scutaria Lamarck individuals exposed to various concentrations of chlorine, but it was difficult to tell if any individuals of this rather hardy species of coral were dead.

Several related studies of the effects of chlorine on both adult and larval corals are necessary before the role of chlorine pollution on coral survival can be fully assessed. The effects on adults and larvae of long-term exposures to low levels of chlorine could be studied if the equipment for regulation of the addition of the chlorine gas or hypochlorite solution were devised. Larval settlement, growth, calcification, etc. could then be followed. Even with short exposures, physiological parameters of health could be studied, such as respiratory rate, nutrient uptake, etc.

Sodium hypochlorite rather than chlorine gas was chosen to make the chlorine solutions in the laboratory experiments although the Kaneohe Sewage Treatment Plant employs the gas.

While a steady-state system with chlorine gas bubbling into seawater would have been the best approximation to the real situation, this was not used due to the difficulties inherent in setting up such a system and the danger involved in working with chlorine gas (Joyner and Durel, 1962; Elmes and Bell, 1963). Furthermore, both chlorine gas and sodium hypochlorite undergo identical reactions in water, and hypochlorite is the main residual (Baker, 1969). The only difference might be in the pH of the solution. However, in the highest concentration of chlorine used in the experiments, 40 mg/l, the pH of the seawater did not change when the hypochlorite was added. However, sewage treatment plants rarely chlorinate to the breakpoint, and thus the chlorine in sewage effluents is mostly in the form of combined residuals (Standard Methods, 1971).

Since the concentration of chlorine present in the solutions dropped with time, the planulae could not be exposed continuously. This was unfortunate because organisms in the field are probably exposed to low levels of chlorine over long periods of time rather than to high concentrations for short periods. Nevertheless, the short exposure times are realistic for pelagic larvae which may drift across a sewer outfall only to be pushed away by the upsurging water or for larvae which may be passed through the cooling system of a power plant.

Even short exposures of larvae to low levels of chlorine were hard to achieve in the laboratory since concentrations up

to 0.06 mg/l dropped to 0.02 mg/l or less in 10 minutes (Table 3). Concentrations below 5 mg/l were especially difficult to mix because of the unpredictable reactions with the seawater and glassware. Waugh (1964) also reported such variability in the preparation of chlorine solutions. The stability of the chlorine solutions during the exposure period could probably have been somewhat improved, however, had they been allowed to sit perhaps 5 minutes rather than one minute before being assayed and used in the experiments.

A comparison of the orthotolidine technique with the iodometric technique revealed that the latter method characteristically gives higher chlorine residual values. Four assays with the orthotolidine method of chlorine solutions mixed identically, and six assays with the iodometric method yielded average chlorine residuals of 3.60 mg/l and 5.00 mg/l, respectively, with standard deviations 0.29 and 0.71, respectively. A similar discrepancy in the methods is also reported in Standard Methods (1971) and Zillich (unpublished). The differential can be especially marked in samples containing a large amount of organic matter; for instance, a difference of 2-5 mg/l is common in settled sewage (Standard Methods, 1971).

In conclusion, chlorine pollution is presently not a local problem in the marine environment. If much higher levels of chlorine are introduced into the seawater, coral planulae will most likely remain unaffected, but the possible fate of most

other marine organisms remains unknown.

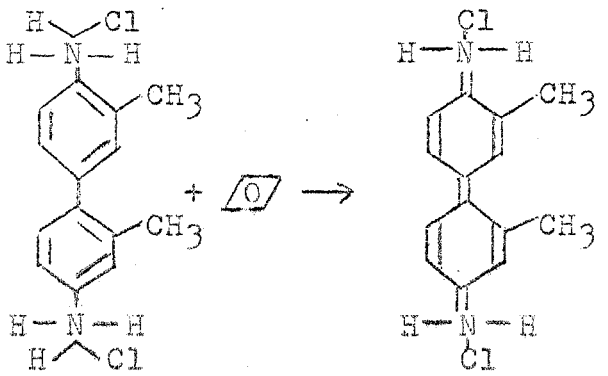
The orthotolidine technique is a well-documented, widely-used colorimetric test for determining the level of total available chlorine in unpolluted or grossly polluted water (Ellms and Hauser, 1913; Theriault, 1927; Adams and Buswell, 1933; Chamberlin and Glass, 1943; Standard Methods, 1971). The comparison of the color produced by reaction of the chlorine and the orthotolidine reagent with the permanent color standards may be made visually, and this is the basis upon which many commercial kits are manufactured for use in swimming pools. Such kits are also commonly used in sewage treatment plants (Reginald F. Young, personal communication). The comparison may also be made on a spectrophotometer for more accuracy. In this case the method is sensitive to concentrations from 0.01 to 10.0 mg/l but is less accurate above 1.0 mg/l.

Four prerequisites must be satisfied for the correct color to be produced. First, the ratio of orthotolidine to chlorine must be at least three to one (Chamberlin and Glass, 1943). Second, the acid in the orthotolidine reagent must be sufficient to produce a pH 1.3 when the sample is added even if 1000 mg/l alkalinity in the form of $CaCO_3$ is present (Chamberlin and Glass, 1943). Third, the sample must be added to the reagent, not vice versa (Chamberlin and Glass, 1943). Fourth, since the test depends upon the oxidizing power of chlorine, other oxidizing agents must not exceed the following levels (Hulbert, 1934; Scott, 1934; Standard Methods, 1971):

ferric ion	300 µg/l
manganic ion	10 µg/l
nitrite ion	100 µg/l.

Allowing the color to develop in the dark minimizes interference by nitrites (Standard Methods, 1971). Other substances that may interfere are organic iron compounds, lignocellulose, and algae (Standard Methods, 1971).

The yellow color is produced when the diamine orthotolidine dihydrochloride is fully oxidized to a holoquinone according to the following equation (Tarvin et al., 1934):



The color due to the presence of free chlorine appears almost immediately while that due to combined chlorine develops more slowly. Interfering substances, such as iron, nitrites, and manganese react even more slowly and may therefore have a different mechanism (Tarvin, et al., 1934). For each temperature there is an optimum time to read the absorbance of the sample before the color due to chlorine fades and the color due to other substances appears.

The production of color is according to Beer's law, that is, the absorption of light is directly proportional to the concentration of chlorine. While temporary color standards may be prepared with chlorine, these break down quickly, and more permanent ones are made with a chromate-dichromate solution in a phosphate buffer (Chamberlin and Glass, 1943; Standard Methods, 1971). In the present study the absorption of light by a chlorine solution for concentrations in the range of 0.01 to 0.10 mg/l was read at a wavelength of 435 mμ with a path length of 10 cm.; in the range of 0.10 to 1.0 mg/l also at 435 mμ but with a path length of 1 cm.; and in the range of 1.0 to 10.0 mg/l at 490 mμ with a path length of 1 cm.

While this method was not specifically developed for use in seawater, this does not appear to present a problem. The acid in the reagent produces a suitable pH as prescribed above when a sample of seawater is added to it. The levels of ferric ion, nitrite ion, and manganic ion in seawater are within the limits given above (Sears, 1961). Furthermore, interferences by materials in seawater may be corrected for by reading the absorbance of the chlorinated sample plus reagent relative to unchlorinated seawater plus reagent. Thus, the absorbance of the reference will be due only to interferences, while the absorbance of the sample cell will be due to interferences plus chlorine. If a sewage outfall sample has natural color or turbidity, the extent to which these contribute to the absorbance value can be determined by

reading the absorbance of the colored and/or turbid sample relative to an aliquot of unchlorinated seawater from the same source as for the reference cell mentioned above.

The orthotolidine-arsenite (OTA) technique for assaying chlorine is very similar to the orthotolidine technique (Appendix A). However, use of the strong reducing agent sodium arsenite permits the differentiation of free available chlorine, combined available chlorine, and interfering substances (Gilcreas and Hallinan, 1944; Hallinan, 1944; Standard Methods, 1971). Sodium arsenite quickly reduces both free chlorine and chloramines but not the interfering substances iron, manganese, and nitrite, thereby preventing the chlorine from reacting with any orthotolidine reagent added subsequently. Since free chlorine characteristically reacts quickly with orthotolidine, and combined chlorine and interfering substances react more slowly (Standard Methods, 1971), addition of the orthotolidine and the sodium arsenite reagents at different times permits the separate measurement of color due to free available chlorine alone (by addition of sample to orthotolidine reagent followed immediately by addition of sodium arsenite), color due to interferences (by addition of sample to sodium arsenite followed immediately by addition of orthotolidine reagent), or color due to total available residual chlorine plus interferences (by addition of sample to orthotolidine reagent). The amount of chloramine in the sample can be determined by the difference between total available residual chlorine and free available residual chlorine. The absorbances of the three solutions are measured on a spectrophotometer. Any natural color or turbidity in the sample is present equally in all

solutions and thus is not a problem.

The timing between the addition of each of the reagents and the measurement of the absorbance is critical. In the determination of free available chlorine, the chloramine may react with the orthotolidine, resulting in a higher value of free chlorine than actually present. This is minimized by cooling the sample being tested for free chlorine to as near 1° C. as possible and by never allowing it to exceed 20° C.

For grossly polluted water, only the distinction between total available chlorine and interfering substances is made in Standard Methods (1971). Thus, the amount of total available chlorine plus interferences is measured by the addition of sample to the orthotolidine reagent, while the amount of interference is measured by the addition of sample to sodium arsenite immediately followed by the addition of the orthotolidine reagent.

The iodometric technique is a well-documented, widely-used titration method for determining the level of total available chlorine in unpolluted or grossly polluted water (Hallinan and Thompson, 1939; Standard Methods, 1971). Chlorine oxidizes iodide in a potassium iodide solution to iodine. This is titrated with sodium thiosulfate using starch as the endpoint indicator. The technique is sensitive to chlorine residuals as low as 0.04 mg/l.

Like the orthotolidine method, this test depends upon the oxidizing power of chlorine, and other oxidizing agents such as ferric, manganic, and nitric ions may interfere. Although a neutral titration minimizes interferences, the acid titration is more accurate. The suggested pH is 3-4 (Standard Methods, 1971).

Chlorine gas dissolved in water exists primarily as hypochlorite ion (Sawyer, 1960).

$$Cl_2 + H_2O = 2\ H^+ + OCl^- + Cl^-$$

When a solution of chlorine in seawater is added to an acidified potassium iodide solution and the pH≐8, free iodine is liberated (Hallinan and Thompson, 1939).

$$Cl_2 + 2\ I^- = I_2 + 2\ Cl^-$$

and

$$OCl^- + 2\ I^- + 2\ H^+ = I_2 + H_2O + Cl^-$$

When this is titrated with a sodium thiosulfate solution,

iodide reappears.

$$I_2 + 2\ S_2O_3^{=} = 2\ I^- + S_4O_6^{=}$$

and

$$7\ I_2 + S_4O_6^{=} + 10\ H_2O = 4\ SO_4^{=} + 14\ I^- + 20\ H^+$$

Starch in the presence of iodine in aqueous solution is blue, and disappearance of the blue color indicates the endpoint of the titration (Standard Methods, 1971).

Manganese dioxide, ferric oxide, and nitrites will also oxidize the iodide as follows (Hallinan and Thompson, 1939):

$$MnO_2 + 2\ I^- + 2\ H^+ = I_2 + MnO + H_2O$$
$$Fe_2O_3 + 2\ I^- + 2\ H^+ = I_2 + 2\ FeO + H_2O$$
$$2\ NO_2^- + 2\ I^- + 4\ H^+ = I_2 + 2\ NO + 2\ H_2O$$

and may be mistaken for residual chlorine.

Although this method is not specifically developed for use in seawater, interferences from substances in seawater may be corrected for by the use of a blank titration with potassium iodide and acid in an unchlorinated seawater sample.

Adams, H. W. and A. M. Buswell. 1933. Orthotolidine test for chlorine. J. Amer. Water Works Assn. 25:1118.

Alderson, R. 1970. Effects of low concentrations of free chlorine on eggs and larvae of plaice, Pleuronectes platessa L. FAO Tech. Conf. Marine Pollution, FIR: MP/70/E-3.

Allen, L. A., N. Blazard, and A. B. Wheatland. 1946. Toxicity to fish of chlorinated sewage effluents. Surveyors (London) 105:298.

_____, _____, _____. 1948. Formation of cyanogen chloride during chlorination of certain liquids, and toxicty of such liquids to fish. J. Hyg. 46(2):184-193.

Arthur, J. W. and J. G. Eaton. Chloramine toxicity to the amphipod Gammarus pseudolimnaeus and the fathead minnow Pimephales promelas. J. Fish. Res. Board of Can. (in press).

Baker, R. J. 1969. Characteristics of chlorine compounds. J. Water Poll. Con. Fed. 41(3):482-485.

Banner, A. H. 1968. A freshwater "kill" on the coral reefs of Hawaii. Hawaii Inst. of Mar. Biol., Univ. of Hawaii, Tech. Rept. 15:1-29.

_____, and J. H. Bailey. 1970. The effects of urban pollution upon a coral reef system. Hawaii Inst. of Mar. Biol., Univ. of Hawaii, Tech. Rept. 25:1-66.

Bathen, K. H. 1968. A descriptive study of the physical oceanography of Kaneohe Bay, Oahu, Hawaii. Hawaii Inst. of Mar. Biol., Univ. of Hawaii, Tech. Rept. 14.

Behrman, A. S. 1968. Water is everybody's business; the chemistry of water purification. Doubleday & Co., Inc., Garden City, N. Y. 229 p.

Brock, V. E., W. Van Heukelem, and P. Helfrich. 1966. An ecological reconnaissance of Johnston Island and the effects of dredging. Hawaii Inst. of Mar. Biol., Univ. of Hawaii, Tech. Rept. 11:1-56.

Burns, R. W. and O. J. Sproul. 1967. Virucidal effects of chlorine in wastewater. J. Water Poll. Con. Fed. 39(11): 1834-1849.

Chamberlin, N. S. and Glass, J. R. 1943. Colorimetric determination of chlorine residuals up to 10 ppm with orthotolidine. J. Amer. Water Works Assn. 35:1065, 1205.

Cooper, M. J. 1966. Destruction of marine flora and fauna in Fiji caused by the hurricane of February, 1965. Pacific Sci. 20(1):137-141.

Coventry, F. L., V. E. Shelford, and L. F. Miller. 1935. The conditioning of a chlorine treated water supply for biological purposes. Ecol. 16:60-66.

Dobson, J. G. 1946. The control of fouling organisms in fresh-and salt-water circuits. Trans. Amer. Soc. Mech. Engrs. 68:247-265.

Doudoroff, P. and M. Katz. 1950. Critical review of literature on the toxicity of industrial wastes and their components to fish. 1. Alkalies, acids and inorganic gases. Sewage and Indus. Wastes 22(11):1432-1458.

Edmondson, C. H. 1928. The ecology of an Hawaiian coral reef. B. P. Bishop Mus. Bull. 45:1-64.

_____. 1929. Growth of Hawaiian corals. B. P. Bishop Mus. Bull. 58:1-38.

_____. 1946. Behavior of coral planulae under altered saline and thermal conditions. B. P. Bishop Mus. Occ. Papers 18:283-304.

Ellms, J. W. and S. J. Hauser. 1913. Orthotolidine as a reagent for the colorimetric estimation of small quantities of free chlorine. Ind. Eng. Chem. 5:915.

Elmes, P. C. and D. Bell. 1963. The effects of chlorine gas on the lungs of rats with spontaneous pulmonary disease. J. Pathol. and Bacteriol. 86(2):317-326.

Enslow, L. H. 1932. Chlorination of sewage for BOD reduction. Sewage Works J. 4(2):252-262.

Estes, N. C. 1938. Chlorination of cooling water. Refin. Nat. Gasol. Mfr. 17:191.

Fair, G. M., J. C. Geyer, D. A. Okun. 1968. Water and wastewater engineering. vol. 2. Water purification and wastewater treatment and disposal. John Wiley and Sons, Inc., NY.

Fairbridge, R. W. and C. Teichert. 1948. The low isles of the Great Barrier Reef: a new analysis. Geogr. J. 111: 67-88.

Fan, P. F. and W. C. Burnett. 1969. Sedimentation. In: Estuarine pollution in the state of Hawaii, Part II: Kaneohe Bay study, Section A, p. 33-48. Water Resources Research Center, Univ. of Hawaii, Tech. Rept. 31, Draft.

Gilcreas, F. W. and F. J. Hallinan. 1944. The practical use of the orthotolidine-arsenite test for residual chlorine. J. Amer. Water Works Assn. 36:1343.

Gill, B. 1970. Observations on the effects of chlorinated town supply water on a colony of brown tree frogs (Hyla ewingi) in Foxton [New Zealand]. Tuatara 18(1): 45.

Goreau, T. F. 1964. Mass expulsion of zooxanthellae from Jamaican reef communities after Hurricane Flora. Sci. 145:383-386.

Green, D. E. and P. K. Stumpf. 1946. The mode of action of chlorine. J. Amer. Water Works Assn. 38:1301.

Hallinan, F. J. 1944. Tests for active residual chlorine and chloramine in water. J. Amer. Water Works Assn. 36:296.

_____, and W. R. Thompson. 1939. A critical study of the thiosulfate titration of chlorine. J. Amer. Chem. Soc. 61:265.

Hamilton, D. H., Jr., D. A. Flemer, C. W Keefe, J. A. Mihursky. 1970. Power plants: effects of chlorination on estuarine primary production. Sci. 169:197-198.

Hays, H., P. R. Elliker, and W. E. Sandine. 1963. Effect of acidification on stability and bactericidal activity of added chlorine in water supplies. J. Milk and Food Technol. 26(5):147-150.

Hirayama K. and Hirano, R. 1970a. 19[illegible]a. Influences of high temperature and residual chlorine on the marine planktonic larvae. Bull. Fac. Fish. Nagasaki Univ., 29 [Jap., Engl. abstr.]

_____, and _____. 1970b. Influences of high temperature and residual chlorine on marine phytoplankton. Mar. Biol. 7:205-213.

Holland, G. A., J. E. Lasater, E. D. Neumann, and W. E. Eldridge. 1960. Toxic effects of organic and inorganic pollutants on young salmon and trout. State of Washington, Dep. Fish. Res. Bull. 5:198-215.

Holstrom, M. V. 1970. Hopkins Marine Station. unpublished.

Hulbert, R. 1934. Chlorine and the orthotolidine test in the presence of nitrite. J. Amer. Water Works Assn. 26:1638.

James, G. V. 1941. Water treatment: A comprehensive treatise on the treatment of water for all purposes and effluents purification. Chemical Publishing Co., Inc., NY. 224 p.

Joyner, R. E. and E. G. Durel. 1962. Accidental liquid chlorine spill in a rural community. J. Occup. Med. 4(3):152-154.

Kaplan, H. M. 1962. Toxicity of chlorine for frogs. Proc. Animal Care Panel 12(5):259-262.

Kott, Y. and H. Ben-Ari. 1967. Chlorine dosage vs. time in sewage purification. Water Res. 1(6):451-459.

Lewis, J. B. 1971. Effect of crude oil and oil spill dispersant on reef corals. Mar. Poll. Bull. 2(4):59-62.

MacKay, A. I. 1915. Corals of Kaneohe Bay. In: Hawaiian Almanac and Annual for 1916, p. 136-139. B. P. Bishop Museum, Honolulu, Hawaii.

Marshall, S. M., and A. P. Orr. 1931. Sedimentation on Low Isles Reef and its relation to coral growth. Great Barrier Reef Exped. 1928-29, Sci. Rept., Brit. Mus., (N.H.), 1(5):94-133.

Martin, R. B. 1938. Chlorination of condenser cooling water. Trans. Amer. Soc. Mech. Engrs. 60:475.

Mayer, A. G. 1918a. Ecology of the Murray Island coral reef. Carnegie Inst. Wash., Pub. 213:3-48.

Mayor, A. G. 1918b. Toxic effects due to high temperature. Carnegie Inst. Wash., Pub. 252:175-178.

_____. 1924a. Structure and ecology of Samoan reefs. Carnegie Inst. Wash., Pub. 340:1-25.

_____. 1924b. Causes which produce stable conditions in the depth of the floors of Pacific fringing reef-flats. Carnegie Inst. Wash., Pub. 340:27-36.

_____. 1924c. Growth-rate of Samoan corals. Carnegie Inst. Wash., Pub. 340:51-72.

McKee, J. E., and H. W. Wolf. 1963. Water quality criteria. 2nd ed. California State Water Quality Control Board, Pub. 3-A:1-548.

Merkens, J. C. 1958. Studies on the toxicity of chlorine and chloramines to the rainbow trout. J. Water Waste Treat. 7:150-151.

Motoda, S. 1939. Submarine illumination, silt content and quantity of food plankton of reef corals in Iwayama Bay, Palao. Palao Trop. Biol Stn. Stud. I:637-650.

Muchmore, D. B. 1970. Hopkins Marine Station. unpublished.

Muller, G. 1967. Value of cocci detection in chlorinated waters. [Ger.; Engl., Fr., Sp. summ.] Arch. Hyg. Bakteriol. 151 (5/6):402-409.

Ott, S. 1970. Hopkins Marine Station. unpublished.

Page-Jones, R. M. 1971. Free chlorine in fish farming experiments using seawater effluents. Progressive Fish-Culturist 33:99-102.

Panikkar, B. M. 1960. Low concentrations of calcium hypochlorite as a fish and tadpole poison applicable for use in partly drained ponds and other small bodies of water. Progressive Fish-Culturist. 22:117-120.

Powell, S. T. 1933. Slime and mussel control in the surface condensers and circulating tunnels. Combustion, N.Y. 4:7.

Rainford, E. H. 1925. Destruction of the Whitsunday Group fringing reefs. Aust. Mus. Mag. 2:175-177.

Rhines, C. F. 1965. Fundamental principles of sewage chlorination. In: 20th Industrial Waste Conf., Lafayette, Ind., 4-6 May, 1965. Proc. Ind. Waste Conf. Purdue Univ. 20(118):673-678.

Rotkis, T. 1970. Hopkins Marine Station. unpublished.

Sawyer, C. N. 1960. Chemistry for sanitary engineers. McGraw-Hill, N.Y. 367 p.

Schreiber, J. R. 1970. Hopkins Marine Station. unpublished.

Scott, R. D. 1934. Effect of iron in the determination of residual chlorine. J. Amer. Water Works Assn. 26:1234.

Scott, W. J., and L. W. Van Kleeck. 1934. Chlorine disinfection of sewage. Sewage Works J. 6(4):784-796.

Sears, M., ed. 1961. Oceanography. Amer. Assn. for the Advancement of Sci., Wash., Pub. 67.

Slack-Smith, R. J. 1960. An investigation of coral deaths at Peel Island, Moreton Bay, in early 1956. Univ. Queensland Papers, Dept. Zoology. 1:211-222.

Snow, W. B. 1952. Biochemical oxygen demand of chlorinated sewage. Sewage and Indus. Wastes. 24(6):689-704.

Standard methods for the examination of water and waste water. 197 Amer. Public Health Assn., Amer. Water Works Assn., Water Poll. Con. Fed. 13th ed., N.Y.

Steffensen, S. W. and N. Hash. 1967. Hypochlorination of wastewater effluents in New York City. J. Water Poll. Con. Fed. 39(8):1381-1402.

Tarvin, D., H. R. Todd, and A. M. Buswell. 1934. The determination of free chlorine. J. Amer. Water Works Assn. 26:1645-1662.

Tate, M. W., and R. C. Clelland. 1959. Nonparametric and shortcut statistics. Interstate Printers and Publishers, Inc., Danville, Ill. 171 p.

Theriault, E. J. 1927. The orthotolidine reagent for free chlorine in water. Pub. Health Rep. 42:668

Tsai, Chu-Fa. 1968. Effects of chlorinated sewage effluents on fishes in upper Patuxent River, Maryland. Chesapeake Sci. 9(2):83-93.

_____. 1970. Changes in fish populations and migration in relation to increased sewage pollution in Little Paxtuent River, Maryland. Chesapeake Sci. 11:34-41.

Tseu, W. S. L. 1952. Seasonal variation in the physical ecology of the ponds at Hawaii Marine Laboratory and the adjacent waters of Kaneohe Bay, Oahu. Master's thesis, University of Hawaii, Dept. Zool. vii + 86 p.

Turner, H. J., Jr., D. M. Reynolds, and A. C. Redfield. 1948. Chlorine and sodium pentachlorophenate as fouling preventives in sea water conduits. Indus. and Eng. Chem. 40: 450-453.

Van Eepoel, R. P., and D. I. Grigg. 1970. Survey of the ecology and water quality of Lindberg Bay, St. Thomas. Water Poll. Rept., Caribbean Res. Inst. 6 p.

Vaughan, T. W. 1919. Corals and the formation of coral reefs. Ann. Rep. Smithson. Inst. 1917:189-238.

Visintin, B. and G. Errera. 1958. The hygienic significance of eels in drinking water and tests of their chlorine resistance with particular reference to the reservoirs of the island of Pantelleria, Italy. /Ital./ Rend. Ist. Super. Sanita. 21 (7/8):737-747.

Waugh, G. D. 1964. Observations on the effects of chlorine on the larvae of oysters Ostrea edulis (L.) and barnacles Elminius modestus (Darwin). Ann. Appl. Biol. 54(3): 423-440.

Wood, E. J. F. 1955. Control of fouling in sea-water cooling systems. Commonw. Engr. March, 1955. 313 p.

Yamada, N. 1960. Effects of free chlorine on the respiration of sea weeds. /Jap./ The aquiculture 7(3):24-28.

Young, R. H. F., and P. L. Chan. 1970. Oahu wastewater treatment plant efficiency. J. Water Poll. Con Fed. 42: 2052-2059.

_____, K. L. Morphew, and N. C. Burbank, Jr. 1969. Water quality study. In: Estuarine pollution in the state of Hawaii, Part II: Kaneohe Bay Study, Section A, p. 68-116. Water Resources Research Center, Univ. Hawaii, Tech. Rept. 31, Draft.