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EFFECT OF ANTIBIOTICS ON SURVIVAL OF CARANGID FISH LARVAE *(Caranx mate)*, REARED IN THE LABORATORY*

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Techniques are described which have proved successful in rearing the larvae of nine species of marine fishes in Hawaii. The results of intensive experiments on larvae of the carangid fish, *Caranx mate*, are presented, with emphasis on the effects of various antibiotics on bacterial populations in rearing containers and on larval survival. Microcopepods, gastropod veligers, and rotifers were found to be suitable foods for *C. mate* larvae, and larval survival was enhanced when the phytoplankter *Chlorella* was added to cultures. Four bacterial genera were identified from natural water samples and from rearing containers in culture situations. *Pseudomonas* was the most abundant genus, *Cytophaga* next most abundant, followed by *Vibrio; Flavobacterium* was identified but uncommon. uncommon.

In sea water containing low initial numbers of bacteria, bacteria increased more rapidly and stabilized at a higher density than in sea water containing higher initial densities. Of a number of antibiotics tested, the most effective in decreasing bacteria in sea water and enhancing hatching success and larval survival were the polymyxins, penicillins, and especially erythromycin. Repeated treatments with the same antibiotic on day 3 of the experiment did not reduce bacterial density or improve larval survival, when compared with a single treatment on day 1. These results are discussed in relation to the mode of action of the antibiotics, and suggestions are made concerning profitable lines of future research.

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INTRODUCTION

Concomitant with increasing attention to marine research and aquaculture has been the interest in techniques for rearing marine larvae, particularly of commercially valuable species. Several marine fish larvae have been reared successfully in the laboratory, especially within the families Clupeidae (e.g. Blaxter, 1962) and Pleuronectidae (Shelbourne, 1964). May (1971) summarizes most attempts to raise marine fish larvae from 1878 through 1969.

Considerable research has been conducted on the aquaculture of fishes belonging to family Carangidae, primarily the pompano (*Trachinotus carolinus*; Fields, 1962; Berry and Iversen, 1967; Moe *et al.*, 1968; Finucane, 1969; Bellinger and Avault, 1970) and the yellowtail (*Seriola quinqueradiata*; Inoue and Tanaka, 1966; Tamura, 1970). The pompano is being reared successfully by private aquaculture concerns although the techniques are not available to the public. Several investigators at the University of Miami have reared a number of tropical marine fish species (e.g. see Houde and Palko, 1970 for techniques) including several species of Carangidae (Charles Mayo, unpublished).

In Hawaii, the carangid *Caranx mate* (= *Atule mate* [Cuvier in Cuvier and Valenciennes] 1883; see Williams, 1958) or omaka is a highly-prized food fish exhibiting similar aquaculture potential. This paper reports results of experiments in which *C. mate* larvae were reared to juveniles. The larval rearing techniques used with this fish were found applicable to other carangids and a few species of other families. We hope that our results can be applied to rearing of other semi-tropical or tropical fish.

Bacteria and food appear to be the most important environmental variables during early development in the laboratory. Two high mortality periods usually occur in early development; (1) at time of hatching and (2) when yolk is completely catabolized and larvae must begin to feed. We found, as did Oppenheimer (1955), that bacteria affect hatching of eggs and that by controlling bacteria with antibiotics, percent hatching is significantly increased. Our initial research has been concentrated on the effect of certain antibiotics. The importance of providing appropriate type and concentration of food to meet energy requirements of larvae during the crititical stage after yolk is catabolized has been demonstrated in several studies (*e.g.* Lasker, 1962, 1965; Lasker *et al.*, 1970). A comprehensive summary of experiments in the feeding of larval fishes is given by May (1970).

This paper includes a description of our larval rearing techniques, general life history and early development of *C. mate*, and results of experiments testing the effects of different types of food, bacteria and anti-

biotics on hatching and survival of *C. mate* larvae. The growth of cultured *C. mate* from egg to adult was reported by Watarai (1973). Detailed descriptions of the embryonic and larval development are being prepared by Miller (ms. in prep.) and additional studies of larval energetics and the effects of temperature, salinity, and food are in progress.

REARING METHODS

The rearing technique outlined below was delineated through a series of multiple factor experiments testing various ranges of variables. Where significant differences are referred to in the results, significance occurs at least at the $P < 0.05$ level. Analyses were performed on a time-sharing computer facility using standard BASIC programs for the statistical techniques of analysis of variance, covariance and regression. The rearing techniques are similar to those used by others in rearing invertebrate larvae (e.g. Loosanoff and Davis, 1963) and fish larvae (e.g. Schumann, in Bardach, 1968; Lasker *et al.*, 1970; Houde and Palko, 1970). Most of our experiments were performed with *C. mate* eggs and larvae. A list of other species also reared with this technique is in Table 1. Small volumes of

TABLE 1

Species of fish reared by described technique at 25 °C and 34–36‰.

Scientific name (Gosline and Brock, 1965)	Common name
Dussumieriidae	
Etrumeus micropus	Herring
Engraulidae	
Stolephorus purpureus	Anchovy
Mugilidae	
Mugil cephalus	Mullet
Apogonidae	
Apogon brachygramma	Cardinal Fish
Carangidae	
Gnathanodon speciosus	Jack
Caranx mate	Jack
Pomacentridae	
Abudefduf sordidus	Damselfish
Abudefduf abdominalis	Damselfish
Tetraodontidae	
Unknown species	Puffer

water in a closed system were used so that quantitative data could be collected. A modification of these techniques to larger volumes of water, usually correspondingly increased survival. Under the conditions below, percent survival of carangid larvae through critical stages to 2 weeks from hatching varied from 1–80%, averaging 40%. Mortality of larvae after 14 days was negligible.

Collection of Eggs

For most experiments, fertilized eggs of *C. mate* were collected from the southern sector of Kaneohe Bay in surface tows with a 1-meter plankton net (303 μ mesh). Immediately after collection, samples were diluted in buckets and aerated. At the Hawaii Institute of Marine Biology Laboratory on Coconut Island in Kaneohe Bay, samples were passed through sieves to remove extraneous plankton. Eggs were concentrated, washed in sterile sea water and resuspended in a few liters of sea water. Eggs were then sorted by hand into beakers of sterilized sea water. The eggs when collected vary in size from 690–750 μ maximum dimension, with a mean of about 720 μ. In this paper, 'day 1' refers to the day on which each experiment was begun, usually corresponding to the day after the eggs were fertilized; subsequent days are numbered accordingly.

Artificial Fertilization of Eggs

In some experiments, ripe females were stripped by hand and eggs collected in glass bowls without sea water (dry fertilization). Sperm from a ripe male was mixed with eggs and the mixture allowed to stand 3–4 min. Fertilized eggs were concentrated in a sieve, washed 5–10 min with sterile sea water and resuspended. The remainder of the procedure was similar to that above. A maximum of 60% fertilization was achieved by this method. Survival was poor, however, and for most experiments described below planktonic eggs were used.

Rearing Containers

For most experiments, straight-sided polyethylene 'utility tubs' (21.6 cm height X 31.7 cm diameter) were used. The tubs were green (Webster International Dictionary, 2nd ed., 1957, p. 540; Color #58, chart B. Chromium Green) and were filled with 12 l of sea water. Covers were made of 1/8 in plexiglass, approximately 36 cm in diameter with a small hole drilled in the center for an air tube.

Filtration

Sea water was filtered through a series of three cellulose Cuno filters (Aquapure water filters; cartridge No. P110; Cuno Engineering Corporation, Meridian, Conn.). The three Cuno filters effectively removed most organisms from the sea water, including most bacteria. Best results were obtained when new filters were used daily.

Sterilization

Filtered water was passed through a unit containing ultraviolet germicidal lamps (G.E.). All apparatus was also sterilized by irradiating under germicidal lamps for approximately 15 min.

Aeration

Water in containers was *gently* aerated by means of a 1-ml disposable pipette placed through the center of the cover and attached to an air pump. Oxygen was monitored in the containers daily and adjustment of the oxygen concentration was made to maintain oxygen at saturation level. Considerable care was exercised to avoid bubbling or moving water too violently, or mortality was greatly increased.

Temperature and Salinity

Most experiments were performed within a temperature range of 23–25°C and salinity range of 34–36‰.

Light

Continuous light was provided uniformly over the surface of containers using G.E. Cool-white and Daylight 40-watt fluorescent bulbs. Light intensity varied from approximately 200–500 ft-c (2160–5400 lux*). A high light intensity was necessary to maintain normal phytoplankton growth in cultures and for optimal feeding of fish larvae.

Antibiotics

Several antibiotics were tested (Table 6). Sea water was treated with antibiotics before eggs were added.

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* 1 lux (lx) = 1 lumen per square meter.

Larval Concentration

100 eggs/12 l sea water (1 egg/120 ml; 8.3 eggs/liter).

Food Type

Wild zooplankton

This consisted primarily of microcopepods; *Oithona simplex, O. nana* and *Paracalanus* sp. (Edwin Bartholomew, pers. comm.). Plankton was collected using a night-light and small centrifugal pump attached to a timer. After plankton had been attracted to the light for 2 h prior to sunrise, the water pump switched on, transferring plankton from the collection net into another aerated, 73-μ net. The plankton was washed with sterile sea water and sorted into size groups using a series of sieves with different mesh size. Plankton normally fed to larvae from days 2–12 was approximately 73–150 μ maximum dimension and composed primarily (more than 90%) of micropepod nauplii.

Newly hatched *Artemia salina* nauplii, supplemented with wild zooplankton (primarily adult micropepods) were fed to fish larvae from day 12–50 (see also Table 3).

Veligers

Female *Littorina scabra* adult were collected from sea walls surrounding Coconut Island during the low tide preceeding high high tide of the day. At this time, male–female pairs could be located, and the larger of the pair was selected, usually being the female. Females were placed in tubs of filtered sea water where they usually aligned along the water–air interface. In the laboratory females with veligers (3–4 days old) usually spawned throughout the month on the low high or high high tide (± 2 h). A greater percent of females spawned during spring tides. Veligers (maximum dimension approx. 120 μ) were collected and treated as the plankton above and fed to fish larvae.

Food Concentration

Microcopepods were added to rearing containers to a final concentration of 5/ml sea water; veligers to a final concentration of 10–30/ml; *Artemia* to a concentration of 1/ml.

Time Fed

Fish larvae were fed daily from two days after hatching, the time eyes were pigmented and just prior to opening of gut and mouth.

Phytoplankton

Survival of fish larvae was enhanced by addition of phytoplankton cells (local sp. of *Chlorella*) approximately 6–8 μ diameter. Sea water was passed through filters (10 μ) and water containing phytoplankton placed in Fernbach culture flasks (2.8 l) to which 1 ml each of Nutrient A and B (Loosanoff and Davis, 1963) was added. Air was continually bubbled into flasks maintained at 24–25°C under constant light (as above). Dense cultures of *Chlorella* developed in about 2 days and were then added to larval rearing containers at the beginning of experiments. New algal cultures were started every 2 days. Old cultures were not used, as they were frequently toxic. *Chlorella* cells were placed in rearing containers to a final concentration of approximately 1.0×10^4 cells/ml. New *Chlorella* was added when necessary to maintain this concentration. Concentration of algal cells was estimated using a hemocytometer or Coulter counter.

Larval Counts

For most experiments each treatment combination had 3–4 replications and larvae surviving in each replication were counted at the end of the experiment. In other experiments, with smaller volumes of water (*i.e.* 1 l) surviving larvae were counted after the critical day. In still other experiments, smaller numbers of larvae were used and survivors counted daily.

METHODS FOR ANTIBIOTIC AND BACTERIA EXPERIMENTS

Preliminary to testing the effect of antibiotics on survival of larval fish, we tested their effect on bacterial growth in sea water only. Nine-hundred ml of sterilized sea water was placed in each 1-l beaker (Pyrex, straight sides), the antibiotic added and beakers covered with plexiglass covers (hole in center). Salinity and temperature were ambient at 34–36‰ and 23–25°C, respectively. Ambient light at water surface was approximately 430 lux (40 ft-c); oxygen, 6.0–8.0 ppm; pH, 8.0–8.2. A series of six beakers was set up, arranged randomly. Four beakers were inoculated with antibiotic, two were controls with no antibiotic. After 48 h, two of the four treatment beakers were reinoculated with the same concentration of antibiotic to determine the effect of reinoculation on bacterial growth. Bacterial colony counts were taken before addition of antibiotic and every 24 h thereafter for four to five days. Table 6 lists antibiotics tested, together with test concentrations used. Initial concentrations were selected on the basis of recommended base-line dosages for human children. After the effectiveness of the antibiotic on bacterial growth in sea water

was determined at this concentration, some were selected for tests with fish eggs and larvae at a wider range of concentrations.

To test effect of antibiotics on larval fish hatching and survival past yolk absorption, larvae were reared as previously described except that volume of sea water and antibiotic type and concentrations were varied. Antibiotics and concentrations tested on larvae are summarized in Table 6. In most tests of the effects of antibiotics on larval hatching and survival, 10 eggs were added to 1-l beakers of sea water, the fish were not fed, and counts of larvae were made daily. These experiments were terminated after all larvae were dead (approx. 5–8 days after hatching, depending on temperature). For several of these experiments bacterial colony counts were also made.

Bacterial colony counts were made on various sea water samples collected from Kaneohe Bay stations, sea water system at Coconut Island and sea water undergoing various treatments such as filtration and ultraviolet radiation.

For counts of bacteria in sea water and culture water containing larval fish, 0.1-ml samples were removed daily with sterile l-ml pipettes for the first 3 days; 0.01-ml samples the remaining days. Other samples (*e.g.* Kaneohe Bay water) were taken with a sterile diposable syringe, capped, and plated into petri plates in 0.5–1.0-ml aliquots. All samples were plated into sterile disposable petri plates containing Difco marine agar (#2216). Plates were incubated at room temperature (24–26°C) for 48 h. The number of bacterial colonies were then estimated using standard microbiological counting procedure.

RESULTS

General Life History and Early Development

C. mate occur in inshore waters of the Hawaiian Islands. They are commonly caught by fishermen in Kaneohe Bay, Oahu, from about March to October. Their distribution during winter months is not definitely known. Breeding continues from late March to late October, with peaks in breeding in May and June. In the laboratory, development from egg to the juvenile stage takes about 48–52 days, by which time the fish have attained a standard length of about 30 mm (Watarai, 1973). Juvenile omaka are most abundant in the fall around floating objects, frequently commensal with jellyfish (Rhizostomata). Sexual maturity in captivity is reached approximately 17–18 months from hatching (24–26°C) at a standard length of about 150 mm. The maximum standard length of adults recorded from Kaneohe Bay is 245 mm.

Table 2 summarizes major developmental stages. Details of the morphology and physiology of development will be given by Miller, Cooney and Santerre (mss. in prep.). Eggs obtained from plankton vary somewhat in size (0.69–0.75 mm in diameter) and developmental stage (6–20 h after fertilization). At 24–25°C eggs hatch approximately 24–25 h after fertilization. Eyes are pigmented by approximately 72 h and mouth and jaws are functional by about 96 h after fertilization. Approximately 3 days after hatching, yolk is about 99% utilized. Larvae are given food on day 2 and feeding is observed by day 3 after hatching. Dry weight decreases as yolk is utilized, followed by an increase after feeding begins approximately 4 days after hatching. If no food is provided most larvae die 6 to 7 days after hatching. The growth rate of larvae to the juvenile stage is described by Watarai (1972).

Effect of Food Type on Survival

Several types of food were tested for their effect on survival of *C. mate* larvae (Table 3). Of those listed, three enabled larvae to survive past the critical period after yolk absorption: microcopepods, veligers and rotifers. Additional experiments testing food types, concentrations and feeding schedules are in progress.

Larvae were fed microcopepods, veligers or mixed microcopepods and veligers for approximately 2 weeks after hatching. After this time larvae were fed *Artemia salina* nauplii supplemented with adult microcopepods.

TABLE 2

Time to selected developmental stages of *Caranx mate*, reared in the laboratory (24–25 °C).

Time* (h-days)	Stage	Approximate mean maximum dimension (mm)
0	Fertilization of egg	0.69–0.75
24–25 h (day 1)	Hatching of larva	2.00
72 h (day 3)	Eyes pigmented	2.40
96 h (day 4)	Gut open, mouth and jaws developed; yolk utilized; feeding begins	2.55
96–144 h (days 4–6)	Critical period; beginning of feeding	2.55
Days 48–52	Juvenile coloration	30
Days 540–550 (18 mos)	Sexual maturity	150

*Hours (days) after fertilization.

C. mate larvae were observed feeding on rotifers (*Brachionus plicatilus*) and survived to day 7 after hatching (40% survival); further experiments with this food type are being performed at the Hawaii Institute of Marine Biology. Larvae did not feed or survive on any other foods listed in Table 3.

TABLE 3

Food types tested on *Caranx mate*, showing feeding response and survival past critical period. Concentration was 5 organisms/ml, except where otherwise indicated.

Food type	Maximum dimension (μ)	Period fed* (days)	Feeding response	Growth–survival (past days 6–7)
Early larval development				
Zooplankton Primarily nauplii of microcopepods *Oithona* and *Paracalanus*	73–150	2–12	+	+
Veligers *Littorina scabra* 10–30/ml	120–130	2–12	+	+
Rotifers *Brachionus plicatilis*	73–150	2–12	+	+
Ciliates *Cyclidium* sp.	20	2–8	?	–
Gymnodinium splendens	50	2–8	–	–
Powdered fish food (Longlife)	100	2–8	–	–
Egg yolk – 1 ml/2 liters (Gerber's)		2–8	–	–
Late larval development				
Artemia salina nauplii	750 × 50	12–50	+	
Zooplankton Primarily adult microcopepods *Oithona* and *Paracalanus*	250–500	12–50	+	

*Number of days after hatching.

Effect of Phytoplankton on Survival

Several experiments revealed higher survival of *C. mate* larvae when local *Chlorella* sp. was added to cultures. The effect of phytoplankton on survival could be due to one or any combination of the following:

(1) Phytoplankton release oxygen, keeping oxygen concentration in cultures at or above saturation.

(2) Phytoplankton remove metabolites which might otherwise reach toxic levels (*e.g.*, ammonia).
(3) Phytoplankton release antibiotic substances, reducing bacterial growth.
(4) Zooplankton survive in cultures longer and provide greater nutrition by feeding on phytoplankton.
(5) *C. mate* larvae obtain some nutrition from phytoplankton.

It would be of interest to perform additional experiments clarifying this point. The *Chlorella* technique and its possible role in larval fish culture were also discussed by Houde at the World Mariculture Society meeting (Houde, 1972).

Effect of Antibiotics on Survival

During early experiments it was found that survival of *C. mate* larvae was increased by treating culture water with antibiotics prior to addition of eggs. Subsequently, experiments were performed attempting to identify bacteria in sea water possibly affecting eggs and larvae, and a number of antibiotics were tested for their effect on density of (1) bacteria in sea water only, (2) bacteria in sea water containing fish larvae, and (3) constituent bacterial genera. Percent hatching and survival of larvae through early development and growth of larvae treated with antibiotics were also recorded. Representative experiments are discussed below.

Interpretation of results must be qualified. Total colony counts may not represent the actual density of bacteria present in the sample. The media used may not be optimal for some species. Methods used are applicable to aerobic bacteria only. Finally, without further experiments to verify pathogenicity, it is not possible to state certainly which species are responsible for mortality. It is possible, however, to correlate bacterial density with percent hatching and survival of larvae; that is, certain antibiotics reduce bacterial density, and those which do also promote hatching success and larval survival.

Identification of Bacterial Genera

Four genera of bacteria were identified from samples originating from a variety of environments. They are *Pseudomonas, Vibrio, Cytophaga* and *Flavobacterium;* all Gram-negative bacteria. Identification was accomplished using the scheme of Shewan *et al.* (1960) and Shewan (1963) for differentiation of Gram-negative bacteria in marine environments. Identification of species was not completed and although two or three forms within each genus were recognized only one species of each genus usually occurred in cultures. Most pseudomonads exhibited green fluorescence,

placing them within Group I of genus *Pseudomonas.* Among yellow-pigmented colonies (*Cytophaga* and *Flavobacterium*), *Cytophaga* was predominant; *Flavobacterium* was relatively uncommon. In one experiment the same genera were identified under anaerobic conditions. They thus appear to be facultative anaerobes. Of the four genera, *Vibrio* and *Cytophaga* were more abundant in the anaerobic situation. Sampling was insufficient to state that strictly anaerobic species were absent, and further experimentation is necessary.

Pathogenicity of Bacterial Genera

Although pathogenic species belonging to the above genera in other regions are documented in the literature (*e.g.* Almeida *et al.*, 1968), pathogenicity of the Kaneohe Bay species was not definitely established by satisfaction of Koch's postulates. A species of *Cytophaga* was identified as the probable cause of an eye disease in juvenile and adult *C. mate.* The disease is characterized by marked degeneration of the snout and eyes. The early stage is exemplified by a film-like white covering over the eyes within 24 h from the time infection is first observed. This is followed by rapid necrosis of eye tissue during the next 24–48 h which subsequently spreads to the surrounding tissue. In conjunction with rapid deterioration of the eyes, lesions are formed on the snout, eventually covering the head of the fish. Death usually occurs within 3–5 days. The disease occurs primarily in stressed fish subjected to handling and low oxygen in captivity. It is possible that the same disease may affect survival of larval *C. mate*, but this is yet to be observed or determined.

Abundance of Bacteria

To compare the abundance of bacteria in laboratory cultures with their abundance in Kaneohe Bay, we collected surface sea water samples from different areas in the bay, Coconut Island lagoon, and the sea water system at Coconut Island (Table 4). The mean number of colonies/ml increases from the channel outside of south Kaneohe Bay (Buoys #20 and #8) toward the shores of south Kaneohe Bay (e.g. Kaneohe Marina). Highest, albeit variable, counts of marine bacteria occur in samples taken from the Kaneohe Marine Base sewage outfall, where variability in counts probably reflects changes in outfall with time. The mean number of colonies/ml decreases as water flows through the sea water system at Coconut Island, from Coconut Island Point (the water intake) through various filtration and ultraviolet treatments. The range in mean number of colonies/ml in the bay samples (except Kaneohe Marine base outfall samples) is approximately 80–930 colonies/ml. Table 4 also shows that filtration is relatively effective in reducing bacteria in the sea water.

TABLE 4

Range and mean of bacterial colony counts (total number of colonies/ml) from sea water samples collected at different locations in Kaneohe Bay and the Coconut Island sea water system.

	Channel buoy #20	Channel buoy #8	Coconut Island point	Marine Base sewage outfall	Kaneohe Marina	Coconut Island lagoon
Range	80–360	140–240	200–1020	480–17150	260–930	430–1050
Mean	150	370	580	11070	620	740

Coconut Island sea water system laboratory outflow line

	Outside point (source)	Storage tanks	Single cuno filter	Double cuno filter	Triple cuno filter	Triple cuno filter and ultraviolet
Range	200–1020	320–400	160–400	2–10	0–5	0
Mean	580	360	240	5	2	0

There is seasonal variation in abundance of bacteria. Samples collected in winter months generally contain fewer bacteria. The number of bacteria apparently depends in part on temperature of the sea water, although other factors probably are involved.

In Kaneohe Bay samples, *Pseudomonas* and *Vibrio* species predominate, comprising approximately 40% and 35% of total colonies, respectively. *Cytophaga* species are about 10–20% of the total and *Flavobacterium*, 5–10%.

In laboratory cultures, the density of bacteria was usually significantly higher than in natural waters, as expected. During experiments, total colony counts usually cycled from about 100–1,000,000 colonies/ml. The maximum number of colonies/ml varied from approximately 100,000–1,000,000 colonies/ml. When one compares these values with the normal range of mean colonies/ml in Kaneohe Bay (80–930), it is apparent that the number of colonies/ml may be 10^3-10^5 higher in cultures.

Not only are total colony counts greater in culture situations, but the relative abundance of constituent genera varies. *Pseudomonas* is the most abundant both in the bay and in cultures, but is relatively far more abundant in cultures. While *Vibrio* is more abundant in bay samples than *Cytophaga, Cytophaga* is the more abundant of the two in cultures. *Flavobacterium* is even more uncommon in cultures than in the bay.

Effect of Ultraviolet Light on Bacteria

The effect of UV light on bacteria in sea water and resulting larval hatching and survival was tested. Algal and food cultures were also treated

with UV in an attempt to reduce their bacterial populations before offering them to larval fish. Table 4 shows that UV light is totally bactericidal when sea water is filtered and then passed through a UV-light unit. To obtain optimal sterilization of sea water, however, precautions must be taken. UV lights should be periodically tested, since they often decline in effectiveness. Sea water should not stand in the UV unit after sterilization, as the bacteria count may greatly increase in a short time. The rate of sea water flow should be adjusted to allow maximal effect. The effectiveness of the UV sterilization unit can be tested periodically by plating out samples from the outflow and making colony counts.

No significant improvement in hatching and survival of *C. mate* larvae resulted from UV sterilization of sea water. In fact, in one experiment, survival was actually significantly poorer in UV-sterilized water. Generally, it appears that unless other deleterious organisms such as fungi are problematic, UV sterilization is unnecessary and possibly undesirable. It is possible that alteration of the normal balance of bacterial genera results in overgrowth of *Pseudomonas* with consequent decreases in larval survival (see also p. 68).

Cultures of phytoplankton, *Artemia* and microcopepods used in feeding larvae often harbor large bacteria populations. The bacteria are significantly reduced by rinsing the algal or food cultures with several changes of

TABLE 5

Effect of ultraviolet light exposure on bacterial counts (total number of colonies/ml) in algal and food cultures*.

Time exposed (min)	Culture	Sample A	Sample B	Mean	Culture growth after exposure
0	*Chlorella*	16000	16000	16000	Yes
1		75	70	72	Yes
3		0	0	0	No
5		0	0	0	No
0	*Artemia*	32000	40000	36000	Yes
1		10000	12000	11000	Yes
3		440	560	500	No
5		300	250	275	No
0	Microcopepods	10000	8500	9250	Yes
1		600	500	550	Yes
3		0	0	0	No
5		0	0	0	No

*900 ml of culture in 1-l glass beakers were exposed. After exposure, 20 ml were removed, diluted to 90 ml and examined for subsequent growth of the organisms.

filtered, sterilized sea water before adding to larval fish cultures. Additional reduction of bacteria is achieved by exposing cultures to 1 min of UV radiation. Results of experiments exposing cultures to UV light (Table 5) show that a 1-min exposure is sufficient to significantly reduce bacteria and 3 min or more is totally bactericidal, except for *Artemia salina* cultures. Exposure of *Artemia* for 3 min effectively reduces, but does not completely eleminate bacteria. None of the organisms tested was able to withstand exposure longer than 1 min. This technique for reducing bacterial contamination in food and algal cultures must be used cautiously to be sure organisms can withstand a 1-min exposure; some species may not survive such treatment.

Growth of Bacteria in Sea Water only

Bacterial colonies were counted from samples removed from sea water controls (see **Methods**). No algae, larvae or antibiotics were added. Fig. 1 shows the increase in density of bacteria for the experimental period. An

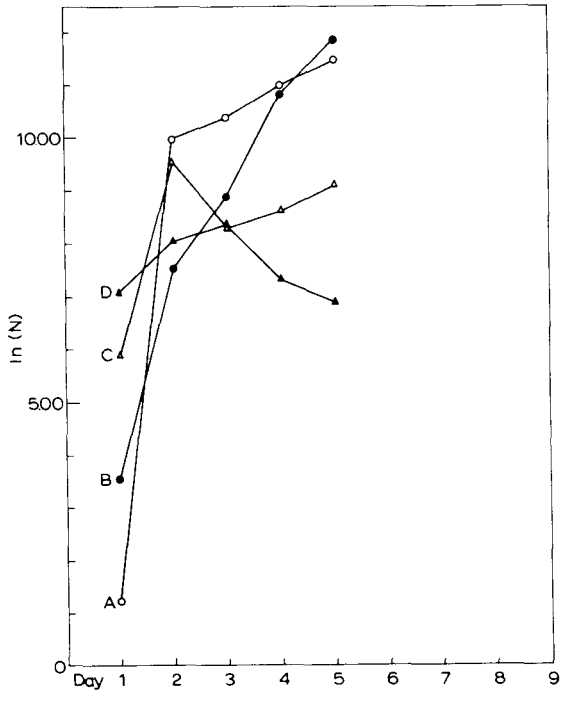

Fig. 1. Change in density of bacteria (all genera combined) in control cultures of sea water only. Y = Mean ln (N), where N = Total number of bacterial colonies/ml (3 replicates); X = Day of experiment. A-Experimental control with low initial number of colonies/ml; B and C-Experimental controls with intermediate initial number of colonies/ml; D-Experimental controls with high initial number of colonies/ml.

interesting feature of bacterial growth in these sea water cultures is that the initial density of bacteria appears to affect subsequent bacterial growth rate. In sea water with lower initial bacterial densities, bacteria rapidly increase to a high density (Fig. 2 A and B). In sea water with higher initial bacterial densities (Fig. 1 C and D) bacteria increase less rapidly, often stabilizing at a lower density than in cultures with lower initial densities. The rapid increase to a high density (in terms of total colonies/ml) probably represents an overgrowth of *Pseudomonas* species, which appear to have a higher growth rate in culture than *Cytophaga* and *Vibrio*. This is discussed further below.

Growth of Bacteria in Sea Water with Fish Larvae

Other experiments, similar to above except for the addition of 10 *C. mate* eggs to each 1-l beaker, show results comparable to those above. The increase in density of bacteria over time is shown in Fig. 2. Again, lower initial densities result in faster bacterial growth. In the experiment

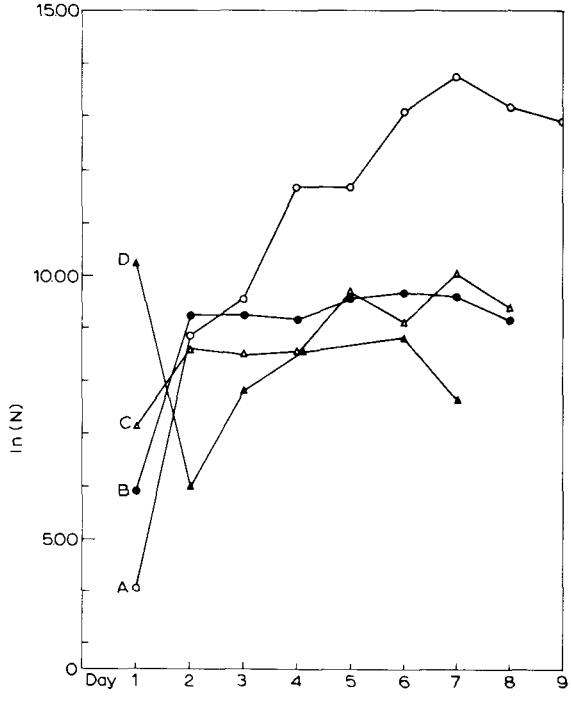

Fig. 2. Change in density of bacteria (all genera combined) in control cultures with *C. mate* larvae. Y = Mean ln (N), where N = Total number of bacterial colonies/ml (3 replicates); X = Day of experiment (= day after fertilization of eggs). A-Experimental controls with low initial number of colonies/ml; B and C-Experimental controls with intermediate initial number of colonies/ml; D-Experimental controls with very high initial number of colonies/ml (high count probably due to leaving sea water in ultraviolet light unit after sterilization; UV light off).

depicted in Fig. 2 D a very high initial bacterial count existed. This resulted from allowing sea water to stand in the UV unit after sterilization with UV light off for some time before sampling. Consequently, *Pseudomonas* increased rapidly, overgrowing *Cytophaga* and *Vibrio.* Percent hatching of eggs was low in this situation; poor hatches generally occurred when the initial total bacterial colony counts exceeded a density of approximately 22 000 colonies/ml (ln 22 000 colonies/ml = 10.000 approx.) on day 1.

An example of relative density of bacterial genera in larval control cultures not treated with antibiotics is given in Fig. 3, where larval survival is also indicated. *Pseudomonas* is most abundant in all these cultures, including the experiment represented, followed in abundance by *Cytophaga* and lastly by *Vibrio. Flavobacterium* was not observed, and was generally rare in laboratory cultures. Considerable mortality occurred in the first 2 days due to reduced hatching. In the typical example of Fig. 3, bacterial density remained relatively stable and mortality was about average for controls (no antibiotic treatment). In other experiments with higher or lower initial bacterial densities, bacterial density tended to fluctuate greatly and mortality was higher.

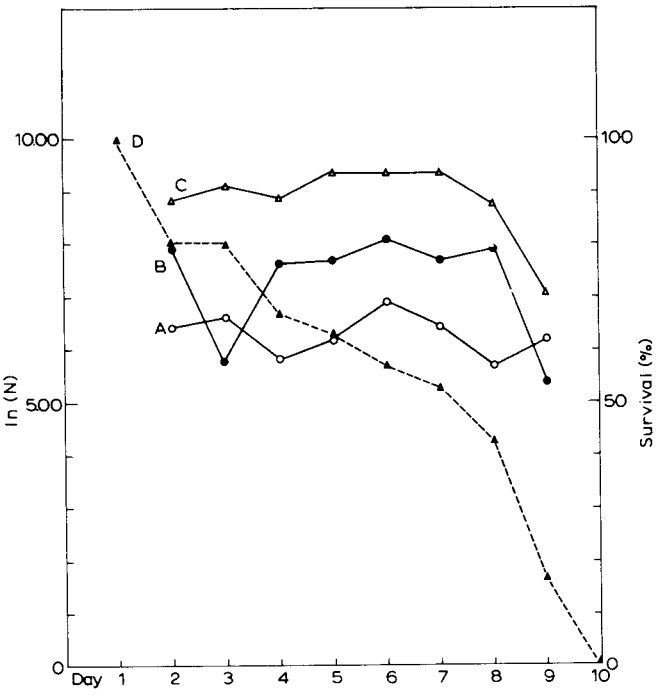

Fig. 3. Change in density of constituent bacterial genera in control cultures of *C. mate* larvae and percent survival of *C. mate* larvae. Left Y-axis = Mean number of colonies/ml (3 replicates); Right Y-axis = Mean percent survival of larvae; X = Day of experiment. A-*Vibrio*; B-*Cytophaga*; C-*Pseudomonas*; D-Mean percent survival of *C. mate* larvae (dashed line).

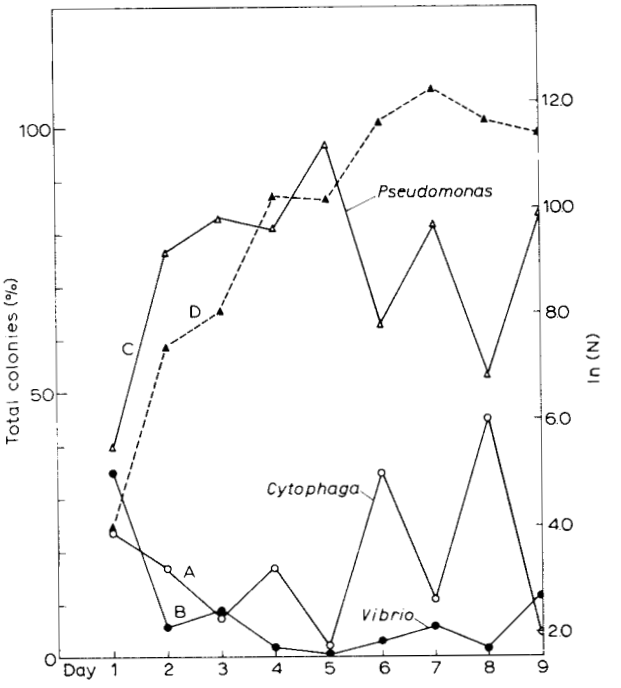

Fig. 4. Cycling density of bacterial genera in terms of percent of total bacterial colonies/ml (solid lines). Total number of bacterial colonies (all genera combined) is indicated by broken line. Left Y axis = Mean percent of total bacterial colonies/ml; Right Y axis = ln (N), where N = Mean number of total bacterial colonies. X = Day of experiment. A-*Cytophaga*; B-*Vibrio*; C-*Pseudomonas*; D-Total.

When counts for all control cultures were combined, *Pseudomonas* colonies comprised an average of 74% of the total colonies; *Cytophaga*, 18%, and *Vibrio*, 6%. In Fig. 4, an example is given of relative density of genera in terms of percent of the total number of colonies/ml per day. The pattern exhibited is the one most frequently observed in cultures, with a daily cycle in abundance of *Pseudomonas* and *Cytophaga*. Initially, all three genera are nearly equivalent in density. As *Pseudomonas* density increases from day 1–2 (Fig. 4 C), *Cytophaga* density decreases (Fig. 4 A). Thereafter, each increase in density of one genus correlates with a decrease in the other. *Vibrio* density in general does not exhibit as definitive a cycle (Fig. 4 B), but tends to interact with density of *Pseudomonas* on certain days. Sea water, prior to use in a culture situation, usually contains the three major genera in approximately equivalent abundance. In culture, the negative correlation of density of *Pseudomonas* with density of *Cytophaga* and perhaps *Vibrio*, suggests that a competitive interaction exists, characterized by cycling abundance of bacterial genera. Finally, after a number of days (depending mostly upon volume of water)

TABLE 6

Antibiotics tested for effect on bacterial growth in sea water and on survival of *Caranx mate* larvae. Antibiotics effective in increasing hatch and survival of larval fish are indicated by asterisk. Antibiotics found toxic to larval fish are indicated by cross (toxic at all conc. tested). (With the exception of some tetracyclines, these antibiotics have not been FDA approved for aquaculture of fish.)

Microbial Derivatives of Eubacteriales

Name of antibiotic	*Aerosporin (Polymyxin B sulfate)	*Coly-Mycin (Polymyxin E, Colistin sulfate)
Derivation	*Bacillus polymyxa*	*Aerobacillus colistinus*
Tested	Sea water and larvae	Sea water and larvae
Concentrations tested (ppm)		
Bacteria in sea water	1, 10, 20, 25, 30, 40, 50, 60 70, 75, 80, 90, 100	80
Larvae	1, 8, 10, 16, 20, 25, 30, 40, 50, 60, 70, 75, 80, 90, 100	1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100
Effect on bacteria in sea water	Suppression of *Cytophaga* spp. No suppression of *Vibrio* spp. beyond day 4. No suppression of *Pseudomonas* spp.	Significant suppression of all bacteria for at least 5 days
Recommended concentration for larvae (ppm)	10–30	10–40
Toxic effects (mortality and/or retarded development)	Over approx. 40 ppm	Over approx. 40 ppm

Comments: Both of these antibiotics are effective in increasing hatch and survival. Together with Ilotycin (erythromycin gluceptate) and Penicillin G, these antibiotics are the most effective tested.

Microbal Derivatives of Actinomycetales

Name of antibiotic	† Chloromycetin (Chloramphenicol)	Garamycin (Gentamicin sulfate)
Derivation	*Streptomyces venezuelae*	*Micromonospora* sp.
Tested	Sea water and larvae	Sea water only
Concentration tested (ppm)		
Bacteria in sea water	50	3
Larvae	25, 50	Not tested
Effect on bacteria in sea water	Some suppression of bacteria	*No* significant suppression of bacteria

TABLE 6 (continued)

Recommended concentration for larvae (ppm)	Not recommended 25 and 50 both toxic	Not tested
Toxic effects	Toxic at 25 and 50	Not tested

Comments: Chloromycetin may be less toxic at lower concentrations and Garamycin more effective at higher concentrations. A wider range should be tested.

Name of antibiotic	*Ilotycin (Erythromycin gluceptate)	Kantrex (Kanamycin sulfate)
Derivation	*Streptomyces erythreus*	*Streptomyces kanamyceticus*
Tested	Sea water and larvae	Sea water only
Concentration tested (ppm)		
Bacteria in sea water	4, 8, 11, 12, 16	15
Larvae	1, 4, 8, 12, 16	Not tested
Effect on bacteria in sea water	Significant suppression of total bacteria count for 6 days at all non-toxic concentrations. All bacteria genera suppressed	No significant suppression
Recommended concentration for larvae (ppm)	10–12	Not tested
Toxic effects	Over approx. 12 ppm	Not tested

Comments: Kantrex concentration of 15 ppm tested on sea water may be too low to affect bacteria colony count. More tests should be made. This antibiotic not tested on larvae.

Name of antibiotic	Streptomycin (Streptomycin sulfate)	Neomycin (Neomycin sulfate)
Derivation	*Streptomyces griseus* and other *Streptomyces* spp.	*Streptomyces fradiae*
Tested	Sea water and larvae	Sea water only
Concentrations tested (ppm)		
Bacteria in sea water	50	200
Larvae	50, 100	Not tested

TABLE 6 (continued)

Effect on bacteria	Some suppression of bacteria for 1 day, then same as control	Only slight suppression of bacteria
Recommended concentration for larvae (ppm)	50 – but streptomycin not very effective in reducing mortality	Not tested
Toxic effects	Toxic at 100	Not tested

Comments: A wider range of Streptomycin should be tested, but indications are that it is relatively ineffective.

Name of antibiotic	† Tetrex (Tetracycline HCl)	† Vibramycin (Doxycycline)
Derivation	Various *Streptomyces* spp.	Synthetic tetracycline
Tested	Sea water and larvae	Sea water and larvae
Concentrations tested (ppm)		
Bacteria in sea water	200	5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100
Larvae	50	Same as sea water
Effect on bacteria in sea water	Significant suppression for 1 day, then same as control	Slight suppression for 5 days
Recommended concentration for larvae (ppm)	Toxic at 50	Toxic at all concentrations
Toxic effects	Toxic at 50	Toxic at all concentrations

Comments: Tetrex and Vibramycin probably toxic at all concentrations.

Microbial Derivatives of Fungi

Name of antibiotic	*Geopen (Disodium carbenicillin)	*Penicillin G (Potassium penicillin G)
Derivation	Semi-synthetic penicillin	*Penicillum notatum* and other *Penicillum* spp.
Tested	Sea water and larvae	Sea water and larvae
Concentrations tested (ppm)		
Bacteria in sea water	100	50
Larvae	10, 50, 100	10, 25, 50, 75, 100

TABLE 6 (continued)

Effects on bacteria in sea water	Significant suppression of bacteria for 5 days then equal to control	Significant suppression for 2–3 days then equal to control
Recommended concentration for larvae (ppm)	10–50	50
Toxic effects	Over approx. 50 ppm	Over approx. 60 ppm

Comments: These two penicillin derivatives, together with Polycillin N, significantly increase hatching and survival through critical period. However, they characteristically decrease bacteria count for only a short period (depending on concentration), thereafter allowing rapid increase to control count level.

Name of antibiotic	*Polycillin N (Ampicillin trihydrate)	*Keflin (Sodium cephalothin)
Derivation	Semi-synthetic penicillin with extended action against gram-negative rods	*Cephalosporium* sp.
Tested	Sea water and larvae	Larvae only
Concentrations tested (ppm)		
Bacteria in sea water	25, 50, 75, 100	Not tested
Larvae	10, 25, 50, 75, 100	50, 100
Effect on bacteria in sea water	Significant suppression 3–4 days then equal to control	Not tested
Recommended concentration for larvae (ppm)	10–25	50
Toxic effects	Over approx. 50 ppm	Toxic at 100

Comments: Further experiments should be made comparing the effectivity of Geopen, Penicillin G, and Polycillin in reducing bacterial counts and increasing hatch and survival. Presently they appear to be equally effective. Keflin at 50 ppm significantly improved hatching and survival of larvae, but an insufficient concentration range has been tested to determine the optimal range and toxity level.

Chemical Anti-microbial Agents		
Name of antibiotic	Furadantin (Nitrofurantoin)	Sulfadiazine (*N'*-2-pyrimidinylsutaniamide)
Derivation	1-[(5-Nitrofurfurylidene)-amino] hydantoin	As above

TABLE 6 (continued)

Tested	Sea water and larvae	Sea water only
Concentrations tested (ppm)		
Bacteria in sea water	7	150
Larvae	50, 100	Not tested
Effect on bacteria in sea water	No significant suppression of bacteria	Actually enhanced bacterial growth (approx. 10^5 times greater than control level)
Recommended concentration for larvae (ppm)	50	Not recommended
Toxic effects	Toxic at 100	Not tested

Comments: Furadantin not as effective in increasing hatching and survival as other suitable antibiotics. However, a wider range should be tested.

densities return to levels approximately the same as they were initially. Further, when the three genera are nearly equivalent in abundance, the total number of bacterial colonies is less. In the light of these results it seems best to limit initial treatment of water to single Cuno filtration so that the abundance of genera is approximately equivalent in the beginning of the experiment and overgrowth of *Pseudomonas* does not occur.

Effect of Antibiotics on Bacteria in Sea Water only

A number of antibiotics were first tested for their effect in decreasing bacteria in sea water. The antibiotics tested, derivation, concentrations and effect on bacteria are summarized in Table 6. Antibiotics tested were selected for a variety of reasons. Some were previously shown to be effective in increasing hatching or larval survival in fishes (*e.g.* Oppenheimer, 1955), others were selected because of comparable derivation or structure. Some were selected because of their effect on human gram-negative bacteria. Of the antibiotics tested, those found effective in decreasing bacteria in sea water cultures were: Aerosporin (polymyxin B sulfate), Coly-Mycin (polymyxin E), Ilotycin (erythromycin gluceptate), Geopen (disodium carbenicillin), Penicillin G, and Polycillin N (ampicillin trihydrate). Sulfadiazine actually enhances bacterial growth, resulting in densities of bacteria approximately 10^5 times greater than controls. Those antibiotics found most effective in reducing bacterial density in sea water were selected for testing at a greater range of concentrations.

Effect of Antibiotics on Hatching and Survival of Larvae

Several antibiotics were tested which significantly increased larval hatching and survival. Of these, the penicillins, polymyxins and erythromycin were the most effective. Some other antibiotics in Table 6 may be effective in reducing bacteria but are toxic to larvae (*e.g.* tetracyclines), and some require further testing at wider ranges of concentrations (*e.g.* keflin). Streptomycin, an antibiotic frequently used in rearing marine larvae (*e.g.* Shelbourne, 1964) only slightly reduced bacteria, and no significant improvement in survival was apparent. The most effective anti-

TABLE 7

Mean percent hatching and survival (of original eggs stocked) past critical period of *C. mate* given different antibiotic treatments. Antibiotics arranged approximately in order of increasing effectiveness. One treatment on day 1 only. Data shown are means of all experiments on the particular antibiotic shown. Number of replicates at least 5, usually more; all percent hatches of larvae given antibiotic treatments shown were significantly different from controls ($P < 0.01$) except for Streptomycin.

Treatment	Antibiotic concentration (ppm)	Mean percent hatching	Mean percent survival (past days 6–7)
Controls (no antibiotic)	–	67	36
Streptomycin	50	66	42
Keflin	50	74	67
Polycillin N	10–25	90	55
Penicillin G	50	85	75
Geopen	10–50	85	77
Aerosporin (Polymyxin B)	10–30	87	85
Coly-Mycin (Polymyxin E)	10–40	94	87
Ilotycin (Erythromycin)	10–12	94	93

biotics are summarized in Table 7 in order of increasing effectiveness in promoting hatching and larval survival. In Table 8 they are classified into groups according to chemical mode of action. Examples selected from these major groups of antibiotics are discussed below [Aerosporin (polymyxin B), Polycillin N (ampicillin), and Ilotycin (erythromycin)] to illustrate the effect of antibiotics on bacterial density and larval survival.

TABLE 8

Group, chemical nature and mode of action of some antibiotics tested. Antibiotics effective in increasing percent hatch and survival of *Caranx mate* designated with asterisk. [Information modified from Evans (1965), Gale (1966), Vol. 16 of the 16th Symposium of General Microbiology (1966), Gottlieb and Shaw (1967), and Garrod and O'Grady (1968) 1].

Group and chemical nature	Nonproprietary name (with proprietary examples)	Empirical formula	Mode of action§
Polypeptides (cyclic polyamides)	Polymyxin B (*Aerosporin)	$C_{56}H_{98}N_{16}O_{13}$	1
	Polymyxin E= Colistin A (*Coly-Mycin)	$C_{53}H_{100}N_{16}O_{13}$	
Penicillins and cephalosporins (water-soluble acids, α-amino-β-lactams fused to sulfur-containing ring)	Benzylpenicillins (*Penicillin G)	$C_{16}H_{18}N_2O_4S$	2
	(*Geopen)		
	Ampicillin (*Polycillin N)	$C_{16}H_{19}N_3O_4S$	
	Cephalothin (*Keflin)	$C_{16}H_{16}N_2O_6S_2$	
Aminoglycosides (water-soluble bases containing amino sugars)	Streptomycin	$C_{21}H_{39}N_7O_{12}$	3
Macrolides (large ring lactones with at least 1 sugar moiety)	Erythromycin (*Ilotycin)	$C_{37}H_{67}NO_{13}$	4
Tetracylines (amphoteric, highly substituted naphthacene derivatives)	Tetracyline (Tetrex)	$C_{22}H_{24}N_2O_8$	4
	Oxytetracyline (Vibramycin)	$C_{22}H_{24}N_2O_8$	
Miscellaneous (*p*-nitrophenylserinol derivative)	Chloramphenicol (Chloromycetin)	$C_{11}H_{12}Cl_2N_2O_5$	4

§ Classification of general modes of action:

Group 1: Affect permeability of bacterial cell membrane causing leakage of substances of small molecular weight. Only antibiotics that kill both resting stages and growing bacteria. Bactericidal and bacteriostatic; rapidly induce cessation of bacterial respiration (especially Colistin).

TABLE 8 (continued)

Group 2: Prevent synthesis of cell wall during bacterial growth so cell lysis occurs (membrane ruptures). Require bacterial growth to act. Bactericidal; long period of contact with bacteria necessary.

Group 3: Inhibition of protein synthesis. Require bacterial growth to act. Bacteriostatic; period of contact with bacteria necessary.

Group 4: Inhibition of protein synthesis. Require bacterial growth to act. Bacteriostatic; but inhibit protein systhesis and growth immediately when bacteria exposed.

Groups 2 and 3 are synergistic. Groups 1 and 4 are sequentially synergistic. Groups 1 and 4 are often antagonistic to groups 2 and 3.

Aerosporin (polymyxin B sulfate) is a polypeptide derived from *Bacillus polymyxa.* Fig. 5 shows the effect of different concentrations of Polymyxin B on density of *Pseudomonas, Cytophaga* and *Vibrio* over the experimental period.

Concentrations of 25, 50, 75 and 100 ppm were tested, but only the effects of 25 and 50 ppm are shown in the figure. Concentrations over 50 ppm are toxic to the fish larvae. Polymyxin B appears to suppress *Cytophaga* most significantly; there was less suppression of *Vibrio* and apparently no suppression of *Pseudomonas* species (Fig. 5). The density of *Pseudomonas* at 25 and 50 ppm is actually higher than the density in controls. The suppression of *Cytophaga* and *Vibrio* apparently results in an overgrowth of *Pseudomonas* species.

Nevertheless, treatment of cultures with 25 and 50 ppm significantly increases hatching survival of *C. mate* larvae (Fig. 6). A concentration of 50 ppm, although resulting in better hatching and survival than controls, may be slightly toxic, retarding development to some degree. Other experiments show that lower concentrations of Polymyxin B (approx. 10–20 ppm) are most effective in increasing hatching and survival, without retarding development (Fig. 7); the slightly higher temperature of Fig. 7 was judged not to have affected the antibiotic results, although yolk absorption and subsequent mortality was earlier.

An increase in percent hatching and survival results from treating larval cultures with Polymyxin B although *Pseudomonas* species are not affected by this antibiotic. This may indicate that *Cytophaga* and/or *Vibrio* are decreasing hatching and larval survival more significantly. Further experimentation is necessary to verify this.

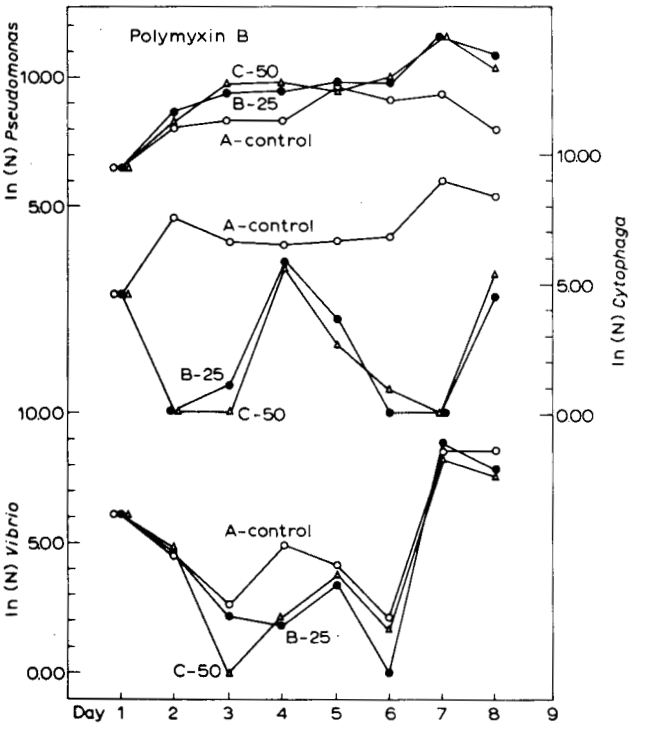

Fig. 5. Changes in densities of *Pseudomonas, Cytophaga*, and *Vibrio* in *C. mate* larval cultures treated with Polymyxin B. Y = Mean ln (N), where N = Number of colonies/ml (3 replicates); X = Day of experiment. A-Control, no antibiotics; B-25 ppm Polymyxin B; C-50 ppm Polymyxin B.

Ilotycin (erythromycin gluceptate) is a macrolide (see Table 8) derived from *Streptomyces erythreus*. Fig. 8 shows the effect of various concentrations of this antibiotic on *Pseudomonas* density. *Cytophaga* and *Vibrio* were suppressed in a similar manner. The high counts observed in this experiment may be due to low initial density; in other comparable experiments with erythromycin far lower counts were observed. Concentrations of 4, 8, 12 and 16 ppm were tested and 16 ppm was found to retard development of larvae.

In spite of the high bacterial densities in this experiment after day 1, Fig. 9 shows that hatching and survival of larvae is significantly improved at 4–12 ppm, perhaps because all genera are suppressed instead of only one or two as with other antibiotics. The optimal concentration (determined from three comparable experiments) is 12 ppm. Erythromycin was generally the most effective antibiotic in increasing both hatching and survival of larvae past the critical stage.

Polycillin N (ampicillin trihydrate) is a semi-synthetic penicillin derivative, a water-soluble acid. Polycillin N is most effective in reducing density

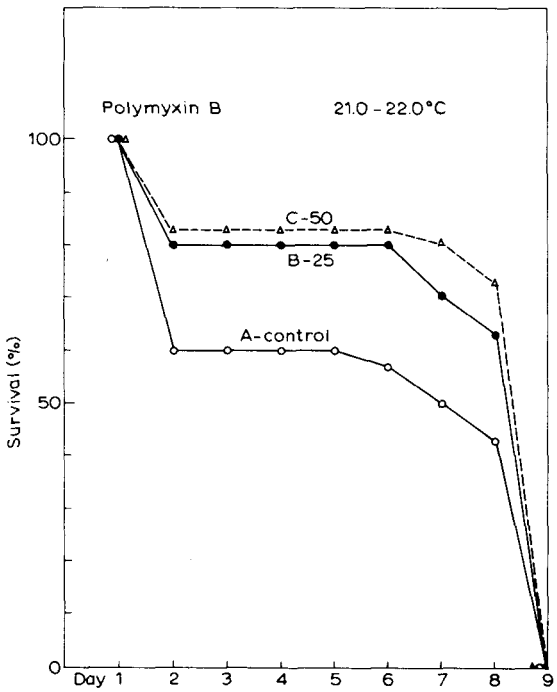

Fig. 6. Percent survival of *C. mate* larvae (not fed) in cultures treated with Polymyxin B (same experiment as Fig. 5) at 21–22 °C. Y-axis = Mean percent survival of larvae (3 replicates); X-axis = Day of experiment. A-Control, no antibiotics; B-25 ppm Polymyxin B; C-50 ppm Polymyxin B. Dashed line indicates survival at a concentration of Polymyxin B which slowed development and may be considered toxic.

of *Cytophaga* (Fig. 10); *Pseudomonas* and *Vibrio* are also suppressed but to a lesser extent. Although concentrations of 25, 50, 75 and 100 ppm were tested, only the results at 25 and 50 ppm are shown; higher concentrations were toxic to larvae. Further, although 50 ppm was effective in reducing bacteria, it appeared to delay larval development slightly. Other experiments indicate that a lower range of approximately 10–25 ppm Polycillin N is maximally effective in increasing hatching and survival of larvae.

Fig. 11 shows the effect of 25 and 50 ppm Polycillin N on the hatching and survival of larvae. Though a significant difference from the control was determined, it can also be seen that erythromycin and polymyxin B (Figs. 7, 9) resulted in better larval survival. Results of other experiments testing penicillin dirivatives showed similar results to those with Polycillin.

Growth of Larvae treated with Antibiotics

Comparisons of growth and development of larvae treated with Polymyxin B, Streptomycin, Erythromycin and Penicillin G with growth of

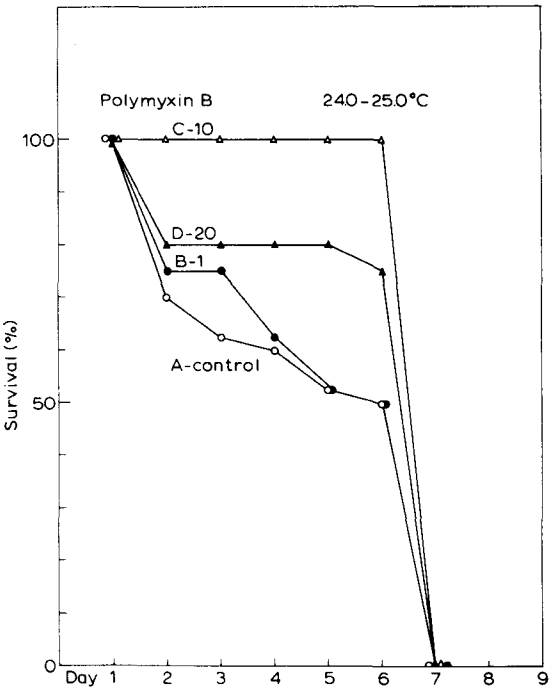

Fig. 7. Percent survival of *C. mate* larvae (not fed) in cultures treated with Polymyxin at 24–25°C. Y = Mean percent survival of larvae (5 replicates); X = Day of experiment. A-Control, no antibiotics; B-1 ppm Polymyxin B; C-10 ppm Polymyxin B; D-20 ppm Polymyxin B.

untreated larvae were made. No significant difference in growth rate was observed at optimal concentrations of these antibiotics. Above these concentrations (see Table 6) retardation in development and growth was often observed.

Number of Antibiotic Treatments

In several experiments, repeated application of an antibiotic was tested on both bacterial density and larval hatching and survival. In most experiments, a treatment was given on days 1 and 3. For most antibiotics, no significant difference in bacterial density or larval survival was noted between those treated on day 1 only and those treated on both days 1 and 3. In a few cases a slightly greater reduction of bacteria occurred with two treatments, but bacterial density usually increased to control levels within a day.

Generally, bacterial populations decreased for the first few days after initial treatment on day 1. The subsequent rate of increase was greatest when initial density was lowest. After a few days bacterial density usually equalled or surpassed the control level: apparently the bacteria developed some resistance to the antibiotic by this time. A second application of the

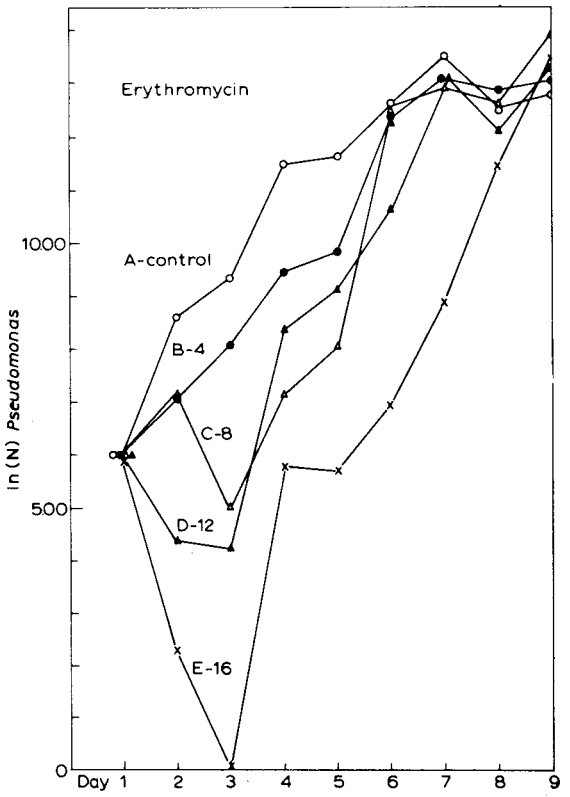

Fig. 8. Change in density of *Pseudomonas* in *C. mate* larval cultures treated with Erythromycin. Y = Mean ln (N), where N = Number of *Pseudomonas* colonies/ml (3 replicates); X = Day of experiment. A-Control, no antibiotics; B-4 ppm Erythromycin; C-8 ppm Erythromycin; D-12 ppm Erythromycin; E-16 ppm Erythromycin.

same antibiotic, therefore, usually had little effect, at least on day 3. Addition of antibiotic again on a later day may prove more effective.

CONCLUSIONS AND DISCUSSION

The major aerobic genera of marine bacteria present in Kaneohe Bay and in laboratory larval fish cultures have been identified. Considerable work remains in identification of species and determination of their relative abundance in different culture situations. Experiments verifying species responsible for *C. mate* larval mortality should be continued. Results so far indicate that a species of *Cytophaga* may be an important pathogen, reducing hatching and survival of larvae. This genus is more abundant in cultures than in the open bay. A species of *Cytophaga* is probably responsible for a degenerative eye disease in juvenile and adult *C.*

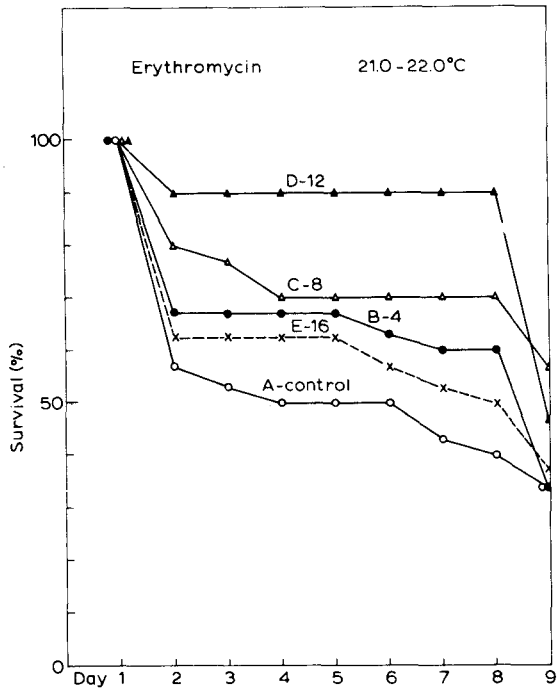

Fig. 9. Percent survival of *C. mate* larvae (not fed) in cultures treated with Erythromycin (same experiment as Fig. 8) at 21–22°C. Y = Mean percent survival of larvae (3 replicates); X = Day of experiment. A-Control, no antibiotics; B-4 ppm Erythromycin; C-8 ppm Erythromycin; D-12 ppm Erythromycin; E-16 ppm Erythromycin. A concentration of 16 ppm (dashed line) was toxic to larvae.

mate. Further, two of the most effective antibiotic groups significantly suppress *Cytophaga* growth in cultures (polymyxins and penicillins), having less effect on *Pseudomonas* and *Vibrio*. The observation, however, that erythromycin (which suppresses all 3 genera) is the most effective of all antibiotics tested suggests that the other genera may contain pathogenic species. Also, high initial colony counts of *Pseudomonas* species are often associated with poor hatches.

The three groups of antibiotics appearing most effective in increasing survival are the polymyxins, penicillins and erythromycin. Most effective is erythromycin. An inexpensive form of erythromycin is available in tablet form, called Maracyn (Mardel Laboratories). The effectiveness of this form is presently being compared against Ilotycin (Eli Lilly & Co.).

The antibiotic streptomycin sulfate has been used frequently in larval culture work (e.g. Loosanoff and Davis, 1963; Shelbourne, 1964). Surprisingly, we found this antibiotic relatively ineffective in reducing bacteria or increasing hatching and survival (Table 7). The reasons for this are

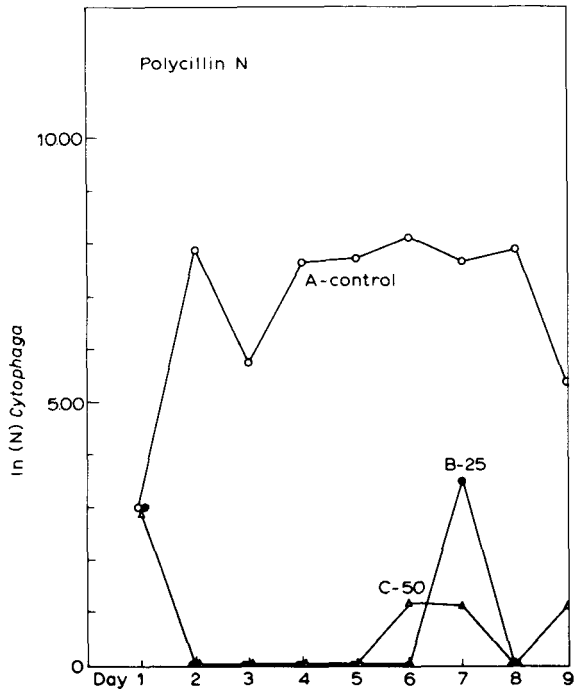

Fig. 10. Change in density of *Cytophaga* in *C. mate* larval cultures treated with Polycillin N. Y = Mean ln (N), where N = Number of *Cytophaga* colonies/ml (3 replicates); X = Day of experiment. A-Control, no antibiotics; B-25 ppm Polycillin N; C-50 ppm Polycillin N.

unclear. Hawaiian species of bacteria may be resistant to streptomycin, or some factor in our culture environment may inhibit the action of streptomycin.

The group 1 antibiotics, or polymyxins, are the only antibiotics which act on bacteria both in resting and growing stages (Table 8). Polymyxins are surface active agents, disrupting the cell membrane; they are bactericidal and bacteriostatic. Group 2 antibiotics, or penicillins, are also bactericidal, but require a longer period of contact with bacterial cells to be effective. They act by inhibiting synthesis of the bacterial cell membrane so that cells eventually lyse. The penicillins require growth of bacteria to be effective. Group 3, or streptomycin, acts on bacteria by inhibiting protein synthesis, altering the conformation of ribosomal RNA. Streptomycin also requires growth at the time of application for effect. This antibiotic is bacteriostatic, bacterial cells continuing to respire after application of the antibiotic although not growing. Group 4 includes several chemical sub-groups: erythromycin, tetracyclines, and chloramphenicol. These antibiotics also inhibit protein synthesis, but unlike streptomycin they act on bacteria almost immediately upon contact. Group 4 antibiotics are also bacteriostatic.

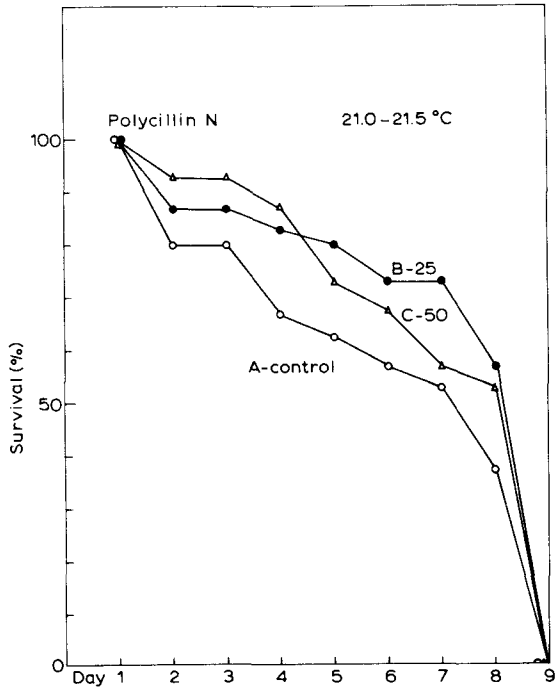

Fig. 11. Percent survival of *C. mate* larvae (not fed) in cultures treated with Polycillin N (same experiment as Fig. 10) at 21–21.5°C. Y = Mean percent survival of larvae (3 replicates); X = Day of experiment. A-Control, no antibiotics; B-25 ppm Polycillin N; C-50 ppm Polycillin N.

It is important to understand the mechanisms by which antibiotics affect bacteria in order to determine which groups are synergistic and can be used in conjunction with one another and which are antagonistic and inhibit the effect of one another. Groups 2 and 3, the penicillins and streptomycin, are synergistic. Both require growth of bacterial cells for effectiveness. Groups 1 and 4 may also be synergistic if used in sequence. Groups which kill bacterial cells on contact (polymyxins) or stop bacterial growth immediately (group 4, e.g. erythromycin) are antagonistic in action to those which require a period of contact with growing bacteria, such as the penicillins and streptomycin.

The application of antibiotics in different sequences might achieve maximum control of bacteria over extended periods, for example, an initial treatment of erythromycin followed by a treatment of polymyxin on about day 5 when bacterial growth again increases. Sequential treatments may solve the problem of bacterial overgrowth of species of a more resistant genus (*i.e. Pseudomonas*) when another genus is suppressed (*i.e. Cytophaga*), such as occurs when cultures are treated with Polymyxin B

(Fig. 5). The combination or sequential use of different antibiotics should have a wider spectrum of effect. Such treatments may also overcome the tendency for bacterial species to become resistant when they are selected by antibiotic application. The rapid rise of *Pseudomonas* at 16 ppm of erythromycin on day 4 (Fig. 8) may be a case in point.

Most antibiotics currently used in the control of marine bacteria in larval cultures were developed for treatment of mammalian diseases, particularly in man, where repeated application at short time intervals is a standard practice. Such antibiotics may not be the most effective in a marine environment. Research on naturally-occurring antibiotics in the sea (*e.g.* produced by marine algae) may reveal more effective antibiotics for control of marine bacteria in laboratory cultures of larvae which may then also require repeated rather than single applications.

In this report techniques were delineated for rearing marine carangid larvae (primarily *C. mate*) in the laboratory. The extension of laboratory results from small volumes of water (closed systems) to mass rearing techniques (open systems) will require modification of present techniques. For example, if larger volumes of continuously flowing sea water are used, application of antibiotics may be complicated and expensive. In this event hatching may be improved by treating eggs with antibiotics before placing them in a mass culture system. Additional antibiotic treatment may be unnecessary if balanced bacterial populations are maintained, as expected in continuously flowing sea water. The provision of food to large numbers of larvae in greater volumes of sea water can be difficult (May, 1970). In Hawaii, where microcopepods and veliger larvae are readily available all year, this problem is not so acute. A rearing system could be designed to automatically pump and sort appropriate wild plankton to feed fish larvae. A method of collecting large quantities of plankton by pumping was used by G.O. Schumann in mass culture of *Scomber japonicus* (described in Bardach, 1968). The design of a mass culture system for carangid larvae should also include means of controlling temperature, salinity, pH, metabolites and light. If a suitable mass rearing system can be designed and maintained economically, it should be possible to cultivate *C. mate* on a commercial scale.

One final obstacle to carangid aquaculture remains. Presently, we are dependent for material upon collection of planktonic eggs from approximately March to October. Future aquaculture of *C. mate* is ultimately dependent upon delineation of techniques for inducing matuaration and spawning of carangids in captivity.

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