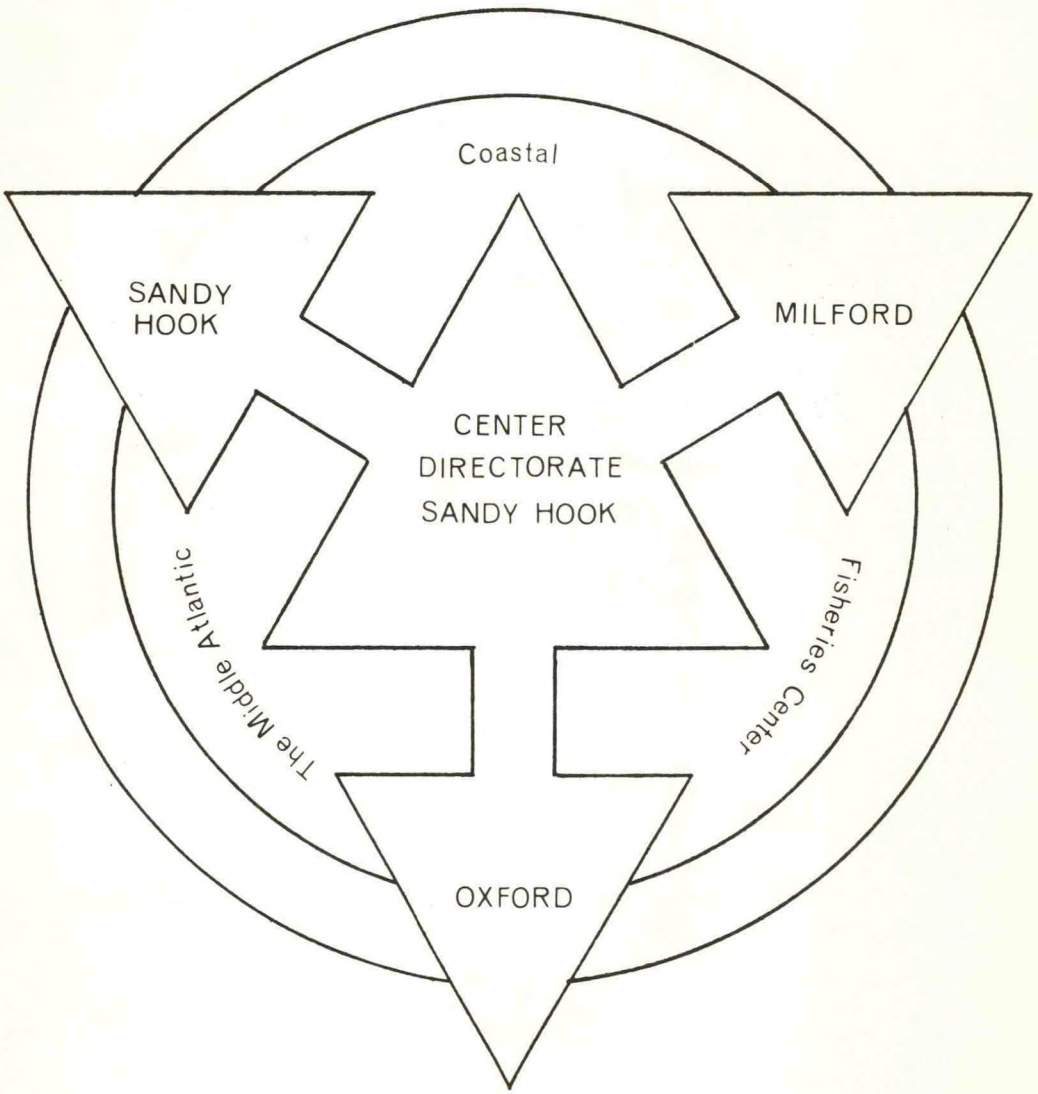
THE ACUTE AND CHRONIC EXPOSURE FACILITY: MILFORD LABORATORY, EXPERIMENTAL BIOLOGY INVESTIGATIONS

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Region

MIDDLE ATLANTIC COASTAL FISHERIES CENTER



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THE ACUTE AND CHRONIC EXPOSURE FACILITY:

MILFORD LABORATORY, EXPERIMENTAL BIOLOGY INVESTIGATIONS

prepared by

Physiological Effects of Pollutant Stress Investigation

Milford Laboratory

Middle Atlantic Coastal Fisheries Center

Milford, Connecticut

August 30, 1973

INTRODUCTION

The purpose of this booklet is to acquaint the members of the Middle Atlantic Coastal Fisheries Center with the acute and chronic exposure facility at the Milford Laboratory. This report contains five main sections:

1. A description of the physical facility
2. A listing of experimental animals available for exposure
3. A listing of accomplishments to date
4. A projection of studies under consideration
5. A listing of publications resulting from cooperative studies involving the exposure facility and other scientific disciplines within the Center.

This briefing is designed not only as a reference for those who have used the services of the facility in the past, but also as a summary for other investigators interested in its potential for cooperative research.

I. PHYSICAL FACILITY

The facility is designed so that a variety of marine organisms can be exposed to sublethal levels of various pollutants. Heavy metals and their effects on marine organisms are being tested at the present time, but plans are being prepared to use pesticides and PCB's in future studies. The exposures are accomplished by short-term (hours to days) static tests and by long-term (weeks to months) continuous-flow tests. At the end of each exposure period, or at intervals during that time, animals are removed and given to investigators for evaluation of the sublethal effects of the test pollutant. The findings to date are summarized in section III of this report.

The major portion of the facility is contained in a 40 x 45 foot laboratory in the lower floor of the main building at Milford. Included in this laboratory are:

A. Nine diluter systems that meter precise amounts of pollutants into 20-gallon all-glass aquaria and 55-gallon fiberglass tanks. Each diluter can be set to deliver 3-5 levels of pollutant to 12 aquaria or tanks. These diluters are the heart of the long-term exposures. They provide six complete changes of water per day and precisely maintain the pollutant level.

B. Glass-working equipment for use in the fabrication and modification of diluter systems, including special gas-oxygen burners, a glass saw, glass cutting lathe for the drill press and a power

borer for making holes in rubber fittings.

C. Compressed air lines with automatic valves to turn air on should the water system fail.

D. A three-tiered platform for static, short-term exposures, equipped with 22 twenty-gallon, all-glass aquaria and 12 sixty-gallon, fiberglass tanks. These are fitted with standard spun-glass and charcoal filters and with compressed-air lines. They may be filled with natural, artificial, or ozonized seawater.

E. A fiberglass sink with six-foot drainboard and high faucet and sprayer for cleaning tanks.

F. Eight 300-gallon tanks with continuously flowing seawater for holding large numbers of animals. This system is plumbed to both the ozonized and natural seawater systems.

G. The diluter systems are presently using heavy metals as test contaminants; however, we have the capability of using other pollutants. Pesticides and PCB's are planned for future work. These pollutants will be removed from the waste water before discharging it into Milford Harbor. A description of the waste treatment facility follows. The Seawater Quality Control Project was given the task of removing or reducing the toxic metal and pesticide load from the diluter systems' effluent. Based on laboratory and prototype studies a waste treatment system was designed to remove 80-95% of the heavy metals and 99% of the

pesticides on a completely automatic basis. The system is based on precipitation and solids separation of heavy metal wastes using commercial-grade caustic soda and centrifugation. Pesticide removal is accomplished by activated carbon adsorption. Seawater containing heavy metals will be collected in P. V. C. pipes and treated with 50% sodium hydroxide and flocculant. The hydroxide and flocculant will cause rapid precipitation and coagulation of heavy metal ions as hydroxides. The seawater precipitate mix will be allowed to settle and then separated in four 100-gallon conical tanks. The seawater will be decanted off and the precipitated, coagulated hydroxide sludge will be separated and strained from the excess seawater by a microstrainer. Sludge will be stored in 55-gallon drums. The strained seawater will be returned to the decanted seawater line and passed through a series of polishing filters that include activated carbon and cloth-wound cartridge types. These filters will be cleaned by freshwater back-flushing on a regular, timed sequence. Finally, the seawater will be sampled and tested for the presence of heavy metal and pesticide carry-over to ensure that all treatment steps are balanced and working at maximum removal efficiency. For pesticide removal, seawater containing these wastes will be segregated from the heavy metal wastewater physically by [illegible] P. V. C. piping. These wastes will be

passed through two 10 ft^3 activated-carbon filter beds for adsorption. Two spare filter beds will be available for treatment upon exhaustion of the primary filters. Spent carbon containing pesticides will be returned to the activated carbon manufacturer for regeneration and removal of all pesticides. The carbon beds will be freshwater back-flushed to remove silt and bacterial buildup. This back-flushing will occur on a timed sequence based on actual flow through each filter bed. The heart of the system, a continuous flow centrifuge, is presently out on bid. This delay in purchasing will cost the system several months of untreated wastewater. An additional $2,000.00 per year operation expenses, including back-flush water, electricity, caustic soda, and alum will be required. In addition, one eight-hour shift for one person per week is necessary to check unit operations and clean out the centrifuge. It is to be noted that this waste treatment system is probably unique in that there are no reports in the literature concerning the removal of heavy metals from seawater on a plant scale.

H. The seawater used in the diluter systems is prefiltered by a 1.7 ft^3 packed-column filter containing large-mesh coconut-shell activated carbon. The water is then ozonized via static mixing, and filtered through 10.2 ft^3 of coconut-shell activated

carbon before storage in a 750-gallon tank. Ozone residual is monitored by an ozone analyzer and bi-weekly total plate counts are made to assure the bactericidal efficiency of the system. Presently, the exposure laboratory is using 10,000 gallons of this ozonized seawater per day. The objectives of this seawater system are:

1. Production of 10,000-14,000 gallons of treated, organic-free water.
2. Removal of all fouling organisms from the seawater.
3. Reduction in the silt load of the raw water which has been up to 15% silt by volume.
4. Elimination of toxic phytoplankton by-products or metabolites which are present during the summer seasons.
5. Reduction in the total bacterial count and removal of all marine pathogenic bacteria.

LABORATORY FLOOR PLAN

A Eight 300-gallon holding tanks

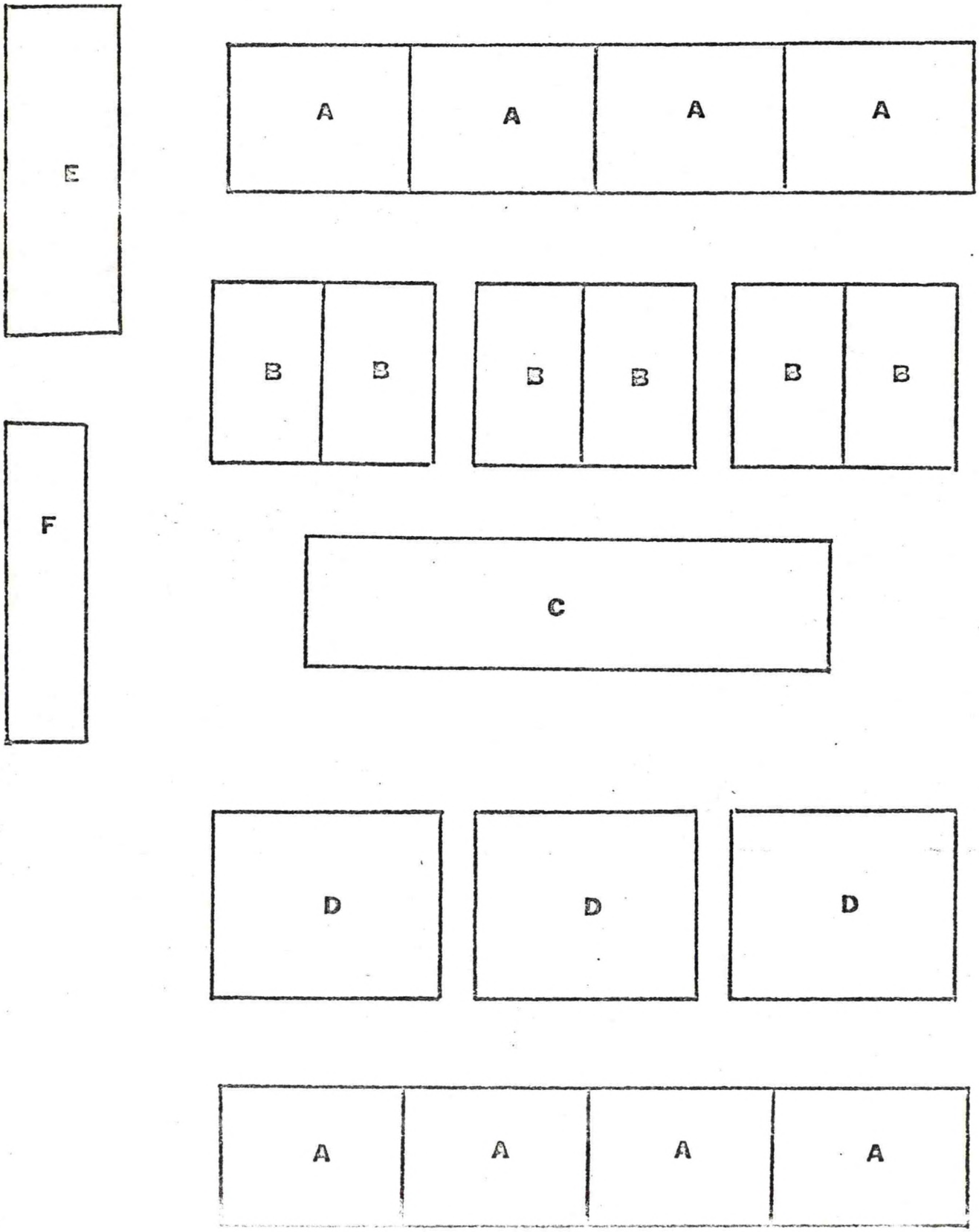
B Six diluters servicing 12 twenty-gallon aquaria each

C Waste treatment

D Three diluters servicing 12 sixty-gallon tanks each

E Three-tiered shelf with aquaria and tanks for static testing

F Sink and wash area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II. ANIMALS AVAILABLE FOR EXPOSURE

This section contains a listing of the adult animals available to the facility for exposure purposes. It lists animals available and in use at the present time, but is not intended to be restrictive. We are willing to work with other animal species provided by cooperating investigators (as we have done with blue crabs supplied by Oxford personnel) and are willing to collect other species found in Long Island Sound. Any and all animal collections are dependent, however, on availability and on sufficient man-hours to accomplish the collection and maintenance of such test animals.

Larval animals are also available in the laboratory and are being used as test animals in the exposure facility. Larval oysters (Crassostrea virginica) and hard clams (Mercenaria mercenaria) are readily available as are the larvae of the slipper limpet, Crepidula fornicata. The Rearing of Bioassay Organisms Task at Milford is developing techniques for rearing a variety of invertebrates so that the effects of contaminants on the entire life cycle or even several generations can be studied. A summary of that work follows:

The objectives of this investigation are (1) to determine the reproductive habits and early development of certain representative species in the Middle Atlantic Bight and (2) to create in the laboratory environments in which these species can be spawned and their embryos, larvae and post-set stages reared in good health. These techniques

will then be made avilable to those who wish to use these organisms in studies of pollution effects.

Because of their long-standing commercial value, much is known of the spawning mechanisms of certain estuarine bivalves and the physiological requirements of their early developmental stages; consequently, the young of these species are already being used in pollution studies in the Center. It is logical then that this investigation start with a study of certain coastal bivalves, such as the sea scallop, the surf clam and the ocean quahog. Initially, the applicability of standard culture methods for estuarine bivalves to the ripening, spawning and culture of the three offshore species will be determined. It is probable that some of the standard methods used for estuarine bivalves can be applied directly but because of the significantly different environment in which the coastal species live, it is likely that new techniques for manipulating certain aspects of reproduction and the culture of the young of the coastal species will have to be developed. It is not necessary that every species selected for preliminary evaluation as a potential study organism prove to be acceptable; for example, of the three coastal bivalves being considered, it appears, at present, that the surf clam is the most amenable to living and reproducing in captivity. If so, it will be the representative species for this class of mollusc in this type of marine environment.

Reproduction and early development in the laboratory of other

classes of marine organisms which have different physiological requirements and which occupy different ecological niches in the Middle Atlantic Bight than do the bivalves will also be evaluated and representative species will be chosen for further study and grooming as study organisms. Major differences in habitat needs, nutritional requirements and reproductive mechanics that exist between dissimilar groups or organisms will undoubtedly require much original research to develop precise culture methods for all the species deemed necessary to rear.

Studies on the role of water quality, the susceptibility of all species selected for study to disease in the admittedly unnatural environment of the laboratory and control of diseases that do occur must accompany these studies if dependable culture methods are to be developed.

Animals in Use at Present

Crustaceans

Carcinus maenas	Green or shore crab
Cancer irroratus	Rock crab
Homarus americanus	Lobster
Eurypanopeus depressus	Mud crab

Molluscs

Mya arenaria	Soft-shell clam
Mercenaria mercenaria	Hard clam
Mytilus edulis	Blue mussel
Crassostrea virginica	American oyster
Nassarius obsoletus	Mud snail
Crepidula fornicata	Slipper limpet

Finfish

Morone saxatilis	Striped bass
Tautogolabrus adspersus	Cunner

III. ACCOMPLISHMENTS TO DATE

This section includes descriptions of work accomplished with the aid of the exposure facility. It includes summaries by various individual projects within the Center and cooperative studies where more than one project was involved.

A. Physiological Effects of Pollutant Stress Task: Milford

1. Physiology Project

a. A study on the effects of copper and cadmium on the oxygen consumption and osmoregulation of two estuarine crabs was completed. Green crabs, Carcinus maenas, and rock crabs, Cancer irroratus, were exposed to various sublethal amounts of copper, as cupric chloride ($CuCl_2 \cdot 2\ H_2O$), and cadmium, as cadmium chloride ($CdCl_2 \cdot 2\text{-}1/2\ H_2O$), for 48 hours. These exposures were conducted at 5 different salinities. At the end of each exposure period, tests of blood serum osmolality and gill-tissue oxygen consumption were performed. Copper-exposed crabs exhibited loss of osmoregulatory function with increasing copper concentration until normally hyperosmotic serum became isosmotic with the surrounding medium. Cadmium elevated green crab serum above its normal hyperosmotic state. Copper had no effect on gill-tissue oxygen

consumption; however, cadmium reduced the rate of oxygen consumption in both species tested.

b. Completion of a study on the effects of cadmium on the gill tissue respiration of the mud crabs, Eurypanopeus depressus. Oxygen consumption rates of gill tissue alone decreased as the cadmium concentration was increased. The mean oxygen consumption rates were as follows: controls, 0.785 µl/hr/mg (SE_M = 0.060); 4 ppm Cd, 0.725 µl/hr/mg (SE_M = 0.064); and 7 ppm Cd, 0.501 µl/hr/mg (SE_M = 0.080). This study was part of a cooperative investigation with the Biology Project.

c. Four bivalve species (Mya arenaria, Mytilus edulis, Crassostrea virginica and Mercenaria mercenaria) were exposed to low levels (below 1 ppm) of silver for 96 hrs. The first phase of the experiment, conducted at 25 ppt salinity, has demonstrated a marked silver-induced elevation in gill-tissue oxygen consumption. This study is being conducted at other salinities at present.

d. A study on the effects of 5 heavy metals on the oxygen consumption and behavior of the mud snail, Nassarius obsoletus, was completed. The condition and oxygen

consumption rates of adult mud snails, Nassarius obsoletus, exposed to five metals (copper, silver, arsenic, cadmium and zinc) and one combination of two metals (copper and cadmium) were determined. The respiratory rate of distressed and retracted snails was found to be lower than the normal rate after exposure to all metals except cadmium which resulted in an elevation of oxygen consumption. The combination of copper and cadmium resulted in a lower rate than either metal alone.

e. The exposure facility was constructed by the Physiology Project and Biology Project.

2. Biology Project: Milford

a. A study on the effects of 11 heavy metals on embryos of the American oyster, Crassostrea virginica, was completed. The acute toxicity of 11 heavy metals to embryos of the American oyster, Crassostrea virginica, was studied and the concentrations at which 50% of the embryos did not develop were determined. The most toxic metals and their LC_{50} values were mercury (0.0056 ppm), silver (0.0058 ppm), copper (0.103 ppm) and zinc (0.31 ppm). Those metals that were not as toxic and their LC_{50} values were nickel (1.18 ppm),

lead (2.45 ppm) and cadmium (3.80 ppm). Those metals that were relatively non-toxic and their LC_{50} values were arsenic (7.5 ppm), chromium (10.3 ppm) and manganese (16.0 ppm). Aluminum was non-toxic at 7.5 ppm, the highest concentration tested.

b. A study on the effects of cadmium on the mud crab, Eurypanopeus depressus, was completed. The results from each of ten tests were averaged and the LC_0, LC_{50} and LC_{100} values were determined. Values for LC_0 and LC_{100} were determined by observation, whereas the LC_{50} value was derived by graphical probit analysis. The LC_0, LC_{50} and LC_{100} values were 1.0 ppm. 4.9 ppm and 11.0 ppm, respectively, with 95% confidence limits for the LC_{50} being 3.9-5.4 ppm. This study was part of a cooperative investigation with the Physiology Project.

c. Tests of five metals (mercury, silver, zinc, nickel and lead), using hard clam embryos as study organisms, were completed. Of the metals tested, mercury and silver were the most toxic. Mercury was 100% lethal at 0.0075 ppm and silver at 0.045 ppm. The estimated LC_{50} value for mercury was 0.0048 ppm, while for

silver it was 0.021 ppm. Zinc and nickel, although not as toxic as mercury and silver, were 100% lethal to clam embryos at 0.25 and 0.60 ppm, respectively, while the estimated LC_{50} values were 0.166 and 0.31 ppm. Lead was the least toxic of the metals tested, although the toxicity was still great in that it was lethal at 1.2 ppm.

d. All exposures noted in this section of accomplishments were set up and maintained by the Biology Project.

3. Biochemistry Project: Milford

Abbreviations used - A/S = acute exposure, static seawater

C/C-F = chronic exposure, continuous-flowing seawater

AAT = aspartate aminotransferase, E.C. 2.6.1.1

NADR-Mg = magnesium-linked nicotinamide adenine dinucleotide reductase

CA^h = carbonic anhydrase, hydratase activity, E.C. 4.2.1.1

CA^e = carbonic anhydrase, esterase activity, E.C. 4.2.1.1

$ATPase^{Na,K}$ = sodium-, potassium-linked adenosine triphosphatase,

E.C. 3.6.1.3

EX = electrophoretic characterization

HCy = hemocyanin

CTF = centrifuged tissue fluid

OSW = ozonized seawater

ASW = artificial seawater

a. A/S Tautogolabrus adspersus-Cd^{2+}: liver AAT, liver NADR-Mg, serum EX

These enzyme systems were investigated in cunners variously exposed to cadmium in ASW for 96 hrs. AAT was significantly higher in controls and NADR-Mg was 10x more sensitive in controls than in cadmium-stressed fish.

b. A/S Cancer irroratus-Cd^{2+}: serum EX (heart AAT, gill CA^h)

The serum of cadmium-exposed rock crab (OSW, 96 hrs.) was characterized electrophoretically for total protein, copper (HCy structure), and peroxidase and phenol oxidase activities (HCy function). Pooled hearts and pooled gills are frozen-stored (-29°C) pending examination for AAT and CA^h activities.

c. A/S Callinectes sapidus-Cd^{2+}: gill CA^e

Preliminary observations were made on the

esterase activity of CA in the gills of cadmium-stressed blue crabs, using a specific inhibitor to identify the enzyme. Results encourage pH studies of the hydratase activity.

d. A/S Mya arenaria-Ag^{2+}: gill EX

Preliminary observations were made on silver-stressed soft-shelled clams, whose gill tissues were characterized electrophoretically for total protein and broad-spectrum esterase activity. Patterns for the esterase (parts of which may be ascribable to CA^{e}) were weaker in the cadmium-stressed gills than in the controls. Again, CA hydratase studies are indicated for future work.

e. A/S Crassostrea virginica-Ag^{2+}: CTF EX

The centrifuged tissue fluid from whole bodies of silver-stressed oysters was characterized electrophoretically for total protein and for copper, and the ratio of fluid volume to wet solids was measured, in an attempt to detect a biochemical response to heavy-metal stress.

4. Chemistry Project: Milford

The chemists have made analyses of water samples and animal tissues. This work has been most useful in

setting up proper exposure levels and in monitoring tissue uptake of heavy metals. This group also made a considerable contribution to the Cooperative Cunner Study.

5. Cooperative Cunner Study: Biology Project, Milford

Physiology Project, Milford

Biochemistry Project, Milford

Environmental Microbiology and Chemistry Investigation, Milford

Pathobiology Investigation, Oxford

This study was designed to determine the short-term (96-hour) physiological response of a local fish, Tautogolabrus adspersus, commonly known as the cunner, to cadmium. A multi-disciplinary approach was used to determine the following: 1) Uptake of cadmium into various tissues and organ systems; 2) Changes in osmoregulation and oxygen consumption rates; 3) Changes in enzymological patterns; 4) Immune response to various antigens; and 5) Induction of histopathological abnormalities. The results have been consolidated into a single manuscript and will be published as an NMFS Special Scientific Report.

6. Mutagenic Effects of Pollutants Investigation: Milford

In conjunction with the Physiological Effects of Pollutant Stress Investigation at Milford, the effects of several heavy metals on the cytogenetics of fertilization, meiosis and cleavage of the oyster are being determined. Such assays ought to be made a regular joint effort of these two investigations.

The basic cytogenetics of the oyster has already been thoroughly studied, so mutagen-induced deviations should be readily recognized. A large amount of experience has further been accumulated now with oyster cytogenetics in a variety of experimental situations.

In an extensive study already completed the mutagenic effects of ionizing radiation on C. virginica were examined (results presented at the 1972 meeting of the Radiation Research Society and written for publication in their journal). This work on ionizing radiation in addition provides a background of expertise on which to base studies of chemical marine contaminants which are potential mutagens and carcinogens.

The Biology Project subjected oyster eggs to 48 different time-metal concentration combinations and made a series of slides of each to show the cytogenetic

effects of heavy metals on meiosis and early cleavage mitosis in the American oyster. After stimulation with sperm which fertilized the eggs as they were spawned, early cleaving eggs were placed in three different concentrations of silver, cadmium, arsenic and manganese. After 15 minutes, 30 minutes and 1 hour, samples of these eggs were fixed for cytogenetic examination. The purpose of this was to estimate the presence, absence and extent of damage to the chromosomes, nuclei and cell division that could be expected to cause lethality, malformations, lowering of vigor and, finally, the sort of genetic change that could be transmitted to the next generation.

In this first study done at Milford, and now fully analyzed, some effects were detected, and, importantly, these were often at a concentration of metal which causes 0% kill. More critical data could have been obtained from analyses of meiotic and fertilization stages, instead of cleavage stages alone, and this should be done next.

Even the lowest concentration of silver - 0% kill - reduced the number of mitosis from an average of about 5 per 1-hour zygote to 0.5. Division irregularities, not including parthenogenetic activities, rose from a control

of 4% to about 25% and remained steady through to a LC_{100} dose. Parthenogenetic activation, which leads to severe inbreeding effects, was as high as 50% at the LC_{100} dose.

Cadmium also reduced the mitotic index of the early stage zygotes, and the LC_0 dose was just as effective in doing so as the LC_{100} dose. Division abnormalities and irregularities were high for the earliest most sensitive time after fertilization even for the LC_0 dose. With cadmium, parthenogenetic activation appeared confused with other abnormalities induced and so could not be properly appraised.

Arsenic did not alter the mitotic index at any dose. Increased evidence of abnormality was not detectable until the latest stage zygotes studied and then only at the LC_{100} dose. At this stage about 50% of the eggs were very grossly abnormal, with nuclei indicative of severe metabolic disturbances.

Manganese did not reduce the mitotic index. However, nuclear and division abnormalities increased 54% over the control for the LC_0 dose, and remained about the same for the LC_{50} and LC_{100} doses for all the timed samples - 15 and 30 minutes and 1 hour.

The abnormals produced by manganese would probably have the most pronounced long-term transmittable genetic effects or congenital effects. Unlike those for the other metals, which, aside from parthenogenesis, were composed of a variety of abnormals whose genetic effects would come about in a circuitous way - the effects noted here consisted chiefly of severe direct changes in the ploidy level of the zygotes, and in losses of pieces of chromosomes, and of whole chromosomes. It seems these effects are the result of disturbances of the spindle in the eggs subjected to manganese. The spindle is the apparatus formed anew for each division of the cell. The spindle apparatus makes possible the orderly, precise division and distribution of the chromosomes to the two new daughter cells.

Statistical analyses were done on these data and analyses confirmed the above interpretations and strengthened them.

An extensive review of literature on genetic effects of heavy metal contaminants has been made. This review has shown that other workers, using higher plants and animals, established some years ago, with certainty, that several of the heavy metals mutate genes. They

break chromosomes and interfere with the course of mitosis and meiosis.

Gonads from different crabs, clams, mussels and Crepidula fornicata exposed to metals for short periods by the Biology Project are now being sampled. Analyses of some of this material have begun. This work for the present will consist mostly in spot-checking. It will be directed toward (1) effects of the contaminants on the gametogenesis and associated genetically critical meiotic stages; (2) effects on cell division and chromosomes that lead to direct genetic effects almost always leading to gamete lethality, semi-sterility or sub-vital mutations.

IV. FUTURE STUDIES UNDER CONSIDERATION

Several studies are under consideration for FY 74. The following proposals demonstrate the value of an exposure facility in the Center's Marine Contaminants Program and emphasize the broad spectrum of investigations involved.

A. Biology Project: Milford

This project will continue to operate the facility and collect and maintain test animals. Studies of the effects of pollutants on living marine organisms will continue and a multi-generation study of the effects of silver on the slipper limpet, Crepidula fornicata, will be conducted. This experiment will determine the sublethal effects of silver on growth, reproduction and respiration.

B. Physiology Project: Milford

The Physiology Project will continue studies of metal-induced changes in osmoregulation and oxygen consumption. We anticipate studies involving cunners, striped bass, lobsters, several crab species and various molluscs. Respiratory studies with embryos and larvae will continue using ultra-microrespirometers. The addition of a multi-channel physiological recorder to our laboratory will enable us to include muscle and heart studies and add new

respiration monitoring techniques. We will also be looking into the intracellular levels of amino acids in molluscs and crustaceans. Such amino acids have been implicated in osmoregulatory processes.

C. Biochemistry Project: Milford

For future work, this project proposes to undertake, with the help of the exposure facility, the following experimentation:

In the short-term study of cadmium-exposed blue crab, Callinectes sapidus, requested of Biology by Pathobiology, gill tissues will be examined for CA hydratase and for sodium-, potassium-linked ATPase activity. The sera will be characterized electrophoretically for total protein and for hemocyanin.

During preliminary tolerance studies of silver-stressed Crepidula fornicata, whole-body homogenates will be examined for AAT, CA hydratase, and ATPase activities, and total-protein electrophoretic patterns will be made. Any responsive biochemical system(s) will be observed during subsequently conducted chronic studies.

Striped bass, Morone saxatilis, will be chronically exposed to cadmium and mercury. The gills will be examined for CA^{h} and ATPase, and livers will be tested

for AAT and NADR-Mg activity.

A variety of crustaceans and molluscs* will be exposed to as yet unselected heavy metals under chronic conditions; gill tissues of all species will be examined for CA^h and, if possible, for ATPase. Crustacean hearts will be examined for AAT and for NADR-Mg. If the northern shrimp, Pandalus borealis, adapts to heavy-metals exposure under chronic testing conditions, its hepatopancreas will be screened for several enzyme activities during tolerance studies, and feasible systems will be observed during subsequently conducted chronic exposures.

D. Environmental Microbiology: Milford

This project proposes to use the exposure facility for one experiment (run in two parts) in which 12 cunners (Tautogolabrus adspersus) will be exposed to 12 ppm $CdCl_2$ for 96 hrs. Another 12 cunners will be held as controls without $CdCl_2$ treatment. The purpose of this experiment is to look for cadmium-induced changes in phagocytic cell metabolism which can be associated with reduced ability to kill bacteria. Such changes include pH changes in phagocytic vacuoles, reduction in lysozyme and reduction in

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*Animals under consideration: Crassostrea virginica, Mya arenaria, Mercenaria mercenaria, Cancer irroratus, Carcinus maenas.

alkaline phosphatase activity.

Also, to use the holding facilities to maintain fish for experiments on antibody production against formalin-killed fin-rot bacteria.

E. Pathobiology Investigation: Oxford

Two studies are proposed: one using striped bass and cunners and the other using blue crabs.

1. Pathological effects of cadmium on the cunner (Tautogolabrus adspersus) and striped bass (Morone saxatilis).

Requirements: It is proposed that in collaboration with the Biology Project the aforementioned fish species will be exposed to 48 and 24 ppm of Cd for varying intervals of time, sacrificed, and undergo excision and fixation of tissues to be examined by light and electron microscopic methods. The details of the proposed experiments are as follows:

A. Owing to their availability and Mr. Newman's previous studies, the cunner will be used in our first set of experiments, the conditions of which will be repeated at a later date when studies utilizing the striped bass are feasible.

B. Twelve healthy fish separated into the following treatment groups will be needed:

Group I - Two fish exposed to 24 ppm Cd for 96 hrs.

Group II - Two fish exposed to 48 ppm Cd for 24 hrs.

Group III - Two fish exposed to 48 ppm Cd for 48 hrs.

Group IV - Two fish exposed to 48 ppm Cd for 96 hrs.

Group V - Four fish maintained in similar environmental conditions as per groups I-IV; however, Cd to be absent as these animals will serve as morphological controls in this experiment.

C. Based upon previous discussion of this work (8 March 1973), it is our impression that we will have the assistance of a particular individual from your laboratory, to whom we can explain and demonstrate the methodologies required for adequate preservation of the recovered tissues -- and who will assume the responsibility for these procedures in our absence or in future experiments. Thus, the

materials and methods will be made available at the onset of the experiment when one of us will be available to participate and instruct in the process of tissue recovery.

2. Histopathological studies of effects of cadmium on the blue crab, Callinectes sapidus.

Preliminary experiments

Crabs will be exposed in 20-gallon tanks in static systems. Four crabs will be placed in each tank, separated from one another by nylon mesh or other such material. For each exposure experiment there will be 8 test animals per concentration of cadmium and 8 control animals for the entire run. In order to bracket the concentration necessary to establish a rough median tolerance limit (or other measurement of toxicity as used by Milford), 5-6 concentrations of cadmium will be used, at least in the first experiment. Assuming six dilutions of cadmium, this means 48 test animals per experiment and 8 controls.

The purpose of the preliminary experiments would be solely to provide an approximate idea of what amount of cadmium is necessary to cause signs of acute toxicity in the blue crab. Histological preparations would not

be made from this series of animals.

Enough crabs should be supplied to run two preliminary experiments -- ones for the second run to be kept in holding facilities at Milford. Each experiment would take 56 crabs. To provide for losses during shipment, acclimation and holding, at least 200 crabs should be provided. Crabs from Chincoteague, already adapted to high salinity, would be the logical choice. The number of experiments necessary is an unknown at this time.

Histological experiments

Following determination of the most satisfactory concentration of cadmium to be used, based on preliminary runs, two experimental series will be set up, to run on consecutive days. Crabs will be held as previously, but 8 test and 8 control animals will be used and only one concentration of cadmium. If possible, equal numbers of mature males and females will be used. Dissection and preservation of tissue samples will be done at Milford at the 96-hour point. The number of crabs per experiment was chosen on the basis of the number that can be dissected and sampled in a single day.

Histopathology at the ultrastructural level may be done depending on the pathology, if any, that is seen by the light microscope. In this eventuality, a second 96-hour exposure series would need to be run.

Chronic toxicity, histological studies

The desirability of studies on chronically exposed animals will rest in large part on data gathered during the acute-toxicity studies.

F. Microbiology: Milford and Fairfield University

The purpose of this study is to determine the numbers and species of marine-occurring yeasts isolated from striped bass which have been exposed to different concentrations of several heavy metal contaminants.

If significant differences in yeast populations are observed, the basic study could be expanded to determine whether these differences are due to the heavy metal concentrations and/or the physiological state of the fish.

Since yeasts are known to constitute a considerable proportion of the microflora of polluted waters, it would be of great value to gather such information, especially factors that govern their numbers.

G. Other Studies

In addition to the above-mentioned proposals, most of the studies reported in the "Accomplishments" section will continue into FY 74. This includes the genetics work, chemistry analyses, and a follow-up to the cunner study. In addition, a number of studies are bound to be initiated as "studies of opportunity".

V. PUBLICATIONS

Calabrese, A.

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Calabrese, A., R. S. Collier, D. A. Nelson and J. R. MacInnes

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Thurberg, F. P., M. A. Dawson and R. S. Collier

Effects of copper and cadmium on osmoregulation and oxygen consumption in two species of estuarine crabs. Submitted to Marine Biology.

Thurberg, F. P., A. Calabrese and R. Greig

The effects of silver on oxygen consumption of bivalves at various salinities. (In preparation).