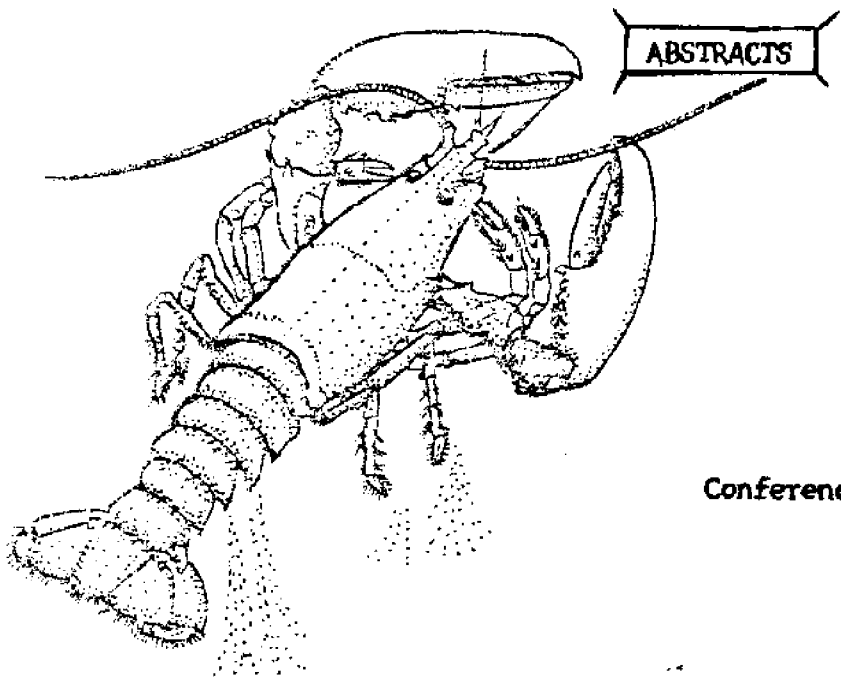
RIU-W-75-001

Sea Grant Lobster Aquaculture Workshop
Technical Session - Recent Advances in Lobster Aquaculture
W. Alton Jones Conference Center
April 21, 1975



Conference Coordinator: J. Stanley Cobb
Department of Zoology
University of Rhode Island
Kingston, R.I. 02881

RECENT ADVANCES IN LOBSTER AQUACULTURE

TECHNICAL SESSION

Date: April 21, 1975 9 A.M. - 5 P.M.

Place: W. Alton Jones Campus, University of Rhode Island

Agenda:

9:00	Welcome and Introduction	
9:10	San Diego State University	
	A. Overview	Ford and Colleagues
	B. Thermal Aquaculture	
	C. Technological Improvements	
	D. Disease	Steenbergen/Schapiro
10:45	Coffee Break	
11:00	Environment Canada, Halifax, N.S. Environment Canada, St. Andrew's, N.B.	
	Molt Control	Aiken
12:00	Lunch	
1:00	University of California at Davis	
	A. Overview	Shleser and Colleagues
	B. Nutrition	
	C. Genetics	
	D. Disease	
	E. Engineering	
	F. Economics	
2:30	Woods Hole Oceanographic Institute	Mitchell
3:00	University of Rhode Island	
	A. Overview	Sastry and Colleagues
	B. Physiological Aspects	
	C. Cannibalism	
	D. Behavior	Cobb
4:30	Boston University	Atema
5:30	Cocktails	
6:30	Dinner. Dinner will be followed by an opportunity for informal discussions.	

TABLE OF CONTENTS

NOTE: THIS PUBLICATION IS OUT OF PRINT

BENEFICIAL USE OF THERMAL EFFLUENT IN LOBSTER CULTURE*

Richard F. Ford, Jon C. Van Olst, James M. Carlberg,
Wayne R. Dorband, and Richard L. Johnson

Department of Biology
San Diego State University
San Diego, California 92182

ABSTRACT

Comparative water quality analyses and rearing experiments were conducted to assess the benefits and problems in using thermal effluent to culture the American lobster, *Homarus americanus*, from the egg to market size. Most of this research was conducted in a special laboratory developed in cooperation with the San Diego Gas & Electric Co. at the Encina Power Plant in Carlsbad, California. Separate studies supported by the Southern California Edison Co. were recently initiated in a similar laboratory at their Redondo Beach, California generating station. These laboratories are supplied with both thermal effluent and ocean quality seawater. Pneumatic mixing valves and electric heating are employed to obtain experimental temperatures. Parallel experiments are being conducted at the Scripps Institution of Oceanography in electrically heated and ocean temperature water.

Salinity, dissolved oxygen, pH, and concentrations of the toxic heavy metals Cu, Zn, Cd, Co, Pb, Cr, and As were essentially the same in thermal effluent from the Encina Power Plant and in ocean water from the outer Agua Hedionda Lagoon and Scripps. Similar results have been obtained in studies recently initiated at the Redondo and Ormond Beach generating stations of the Southern California Edison Co. Analyses of whole animals, muscle, hepatopancreas, and other tissues indicate that concentrations of the heavy metals were relatively low in all life history stages and not significantly different for individuals held in the different water types. These results suggest that chemical toxicity is not a problem in using thermal effluent from the Encina Power Plant.

---

*This work was supported by several agencies. Research at the Encina and Scripps Institution laboratories was sponsored by the NOAA Office of Sea Grant, Department of Commerce, under Grant USDC 04-3-58-122, by a grant from Homarus, Incorporated, and by the San Diego Gas & Electric Company. Work at the Redondo Beach laboratory and some aspects of research at the Scripps laboratory were sponsored by the Research and Development Program of the Southern California Edison Company under contracts U2603906 and U2384902.

larvae of *H. americanus* probably are the stages most sensitive to environmental factors associated with thermal effluent. Thus, they were employed in comparative bioassays of effluent water quality. The larvae were cultured individually and in mass rearing systems at constant temperatures of 18.5 and 21.6°C. Under these conditions, sibling larvae reared to Stage IV in the Encina Power Plant effluent and water from the two ocean sources did not differ significantly in survivorship or size attained. Similar results were obtained in comparative experiments, using the same water sources, to assess the effects of effluent on larvae and juveniles reared to Stage VIII over a three month period at a constant temperature of 22.1°C.

In order to assess the combined effects of temperature and water chemistry, juveniles were cultured individually and in mass rearing systems in effluent at varying temperatures ($\bar{X}$ = 22°C) and in lagoon water at ambient temperatures ($\bar{X}$ = 19°C). The juveniles reared individually in effluent for ten months had significantly higher survival and attained a mean size 11.6 percent larger than did those in lagoon water. Juveniles cultured in mass rearing systems for six months attained a mean size 15.5 percent larger than those in lagoon water.

These preliminary results indicate that thermal effluent may provide a useful and economical source of warm water for lobster culture. Other similar studies are now underway at the Scripps, Encina, and Redondo Beach laboratories.

ES 3

EFFECTS OF SUBSTRATE TYPE AND OTHER FACTORS ON THE GROWTH, SURVIVAL, AND CANNIBALISM OF JUVENILE *Homarus americanus* IN MASS REARING SYSTEMS*

Jon C. Van Olst, James M. Carlberg, and Richard F. Ford

Department of Biology
San Diego State University
San Diego, California 92182

ABSTRACT

Mass rearing experiments were conducted as part of our effort to develop effective techniques for commercial culture of the American lobster, *Homarus americanus*, from the post-larval stage to adult market size. Egg-bearing females received from Massachussetts were held in filtered, flowing seawater at a temperature of 24±1°C until hatching. The young were reared through the four larval stages in Hughes culture units at densities of approximately 3,000 larvae per unit. The larvae were fed live, adult brine shrimp and attained fourth molt stage in 10 days at 24±1°C. They were then transferred into mass-rearing tanks receiving water of ambient San Diego ocean temperatures and were fed frozen, adult brine shrimp daily.

Preliminary experiments on the effects of food dose level and stocking density in mass rearing systems are described. An experiment was conducted to determine the effects of substrate or shelter type on growth, survival, and cannibalism in juveniles. Fourth-stage larvae were stocked at densities of 210-317/m^2 in mass rearing tanks with four different substrate types: short lengths of PVC tubing, small rocks, oyster shell, and sand. After six months, the mean number of survivors per square meter and the mean carapace length for lobsters held in each substrate were: PVC tubes, 22/m^2, 14.4 mm; rock, 24/m^2, 13.9 mm; oyster shell, 30/m^2, 15.0 mm; sand, 6/m^2, 17.3 mm. Mortalities in these tanks were attributed primarily to cannibalism. The loss of one or both claws of the lobsters cultured under different substrate conditions was used as a measure of the degree of fighting and social interaction. Based on the survivorship data, oyster shell appears to be the best culture substrate of those considered for reducing cannibalism and assuring high carrying capacity. Growth rates and aggression, as measured by claw loss, were found to be similar in all substrates tested.

---

* This work is the result of research sponsored by the NOAA Office of Sea Grant, Department of Commerce, under Grants USDC 2-35208 and USDC 04-3-58-122.

DEVELOPMENT AND EVALUATION OF MASS REARING SYSTEMS FOR THE CULTURE OF *Homarus americanus*

Jon C. Van Olst and James M. Carlberg

Department of Biology
San Diego State University
San Diego, California 92182

ABSTRACT

Our initial research on survivorship and growth of lobsters held in communal rearing systems indicated that this method of culture may be economically employed during the early juvenile stages of growth. Presently, research is concerned with the development of mass-rearing culture systems which are easily harvested and relatively non-fouling. Substrates under evaluation include plastic tube "condominiums," strips of corrugated fiberglass, PVC trickling filter media, and plastic honeycomb, as well as our controls of oyster shell and sand bottoms. We are also beginning more detailed experiments to analyze the effects of stocking density, substrate density, temperature, photoperiod, and feeding level on survivorship and growth. The results of this research will be incorporated into a final design for a prototype mass-rearing production tray which we plan to incorporate into a comprehensive scheme for pilot production of lobsters. With the support of the Southern California Edison Company, we hope to construct the pilot scale farm in northern Los Angeles County.

055

DEVELOPMENT AND EVALUATION OF ARTIFICIAL FEEDS FOR CULTURING THE AMERICAN LOBSTER, *Homarus americanus**

Thomas M. Carlberg, Jon C. Van Olst, and Richard F. Ford

Department of Biology
San Diego State University
San Diego, California 92182

ABSTRACT

Food evaluation studies were conducted at the San Diego State University aquaculture laboratories to compare growth and survival of juvenile *Homarus americanus* cultured on diets of both natural or artificially prepared foods. Also employed in these studies were experimental shrimp rations thought to be deficient in some essential micronutrients, which were supplemented with whole animal tissue of the pelagic red crab, *Pleuroncodes planipes*, an inexpensive source of crustacean meal.

Juvenile lobsters were reared individually in compartmentalized trays from Stage IV to an age of one year at ambient southern California seawater temperatures ($\bar{X}$ = 17.2°C). The rates of growth for lobsters fed a daily diet of natural foods (frozen adult brine shrimp, ground whole lobster, or red crab) were superior to growth rates achieved by juveniles fed experimental shrimp rations currently available from Ralston Purina Company and from the Department of Food Sciences at Louisiana State University. One notable exception to these findings was that lobsters reared on a diet of Purina Marine Ration 20, developed for shrimp culture and which we supplemented with fifty percent red crab, exhibited rates of growth nearly equal to those fed frozen brine shrimp or lobster tissue. Another interesting result was that this supplemented ration produced more rapid growth than did either of its two components when presented separately. This seemed to be caused by more rapid fouling due to poorer water stability of these foods compared with those in artificially pelletized form.

---

*This work was supported by several agencies. Research at the Encina and Scripps Institution laboratories was sponsored by the NOAA Office of Sea Grant, Department of Commerce, under Grant USDC 04-3-58-122. Work at the Redondo Beach laboratory and some aspects of research at the Scripps laboratory were sponsored by the Research and Development Program of the Southern California Edison Company under contracts U2803906 and U2384902.

Evaluation of methods for assessing growth showed that measurement of carapace length at molt provided a more accurate estimate of maximum rate of growth than did average length determined from periodic survey data. It also appeared that the duration of experiments at this mean temperature of 17°C should exceed 120 days to provide a meaningful comparative evaluation of food types and their effects on growth.

Given current prices of Marine Ration 20 (20¢/lb.) and red crab (24¢/lb.) and a conversion ratio for *H. americanus* of approximately 4:1, the food cost of producing a one-pound lobster might be less than $1.00.

Studies now in progress at San Diego State University are designed to determine the minimal supplementation necessary to meet the nutritional requirements now lacking from artificial diets and to reduce the dependence on a single fishery such as *Pleuronectes* to provide the supplement. Techniques of using the fish oil extraction method to remove carotenoids from red crab and subsequently adding it to commercial pellets are under investigation. Protein analyses of all of our test rations have been made.

056

EFFECTS OF CONTAINER SIZE ON GROWTH AND MORTALITY OF JUVENILE *Homarus americanus* CULTURED IN INDIVIDUAL REARING SYSTEMS*

Jon C. Van Olst, James M. Carlberg, and Richard F. Ford

Department of Biology
San Diego State University
San Diego, California 92182

ABSTRACT

High levels of cannibalism have been observed in lobsters held in communal rearing systems (Van Olst, Carlberg, and Ford, ms. in press). Most investigators have come to the conclusion that if lobster farming is to become a reality, the animals will need to be held in individual rearing containers in order to avoid the problem. This single-cell rearing approach will be costly and difficult to engineer. To minimize equipment and water treatment costs, the containers should be as small as possible, without unduly restricting the growth or survivorship of the lobsters. Some restriction in growth rate may be tolerable if adequate cost benefits are realized. Our approach will be to weigh the savings in facility and water treatment costs against the losses from decreased growth and higher mortality in a series of cost-effectiveness equations.

Although we have conducted two prior experiments on the effects of container size and confinement stress, we did not feel that the results were adequate. In order to provide more reliable data for the cost analysis, a rather comprehensive laboratory experiment is now underway. A series of rectangular individual rearing containers ranging from 7 cm^2 to 745 cm^2 in bottom area were constructed of plexiglass, covered with fiberglass screen on the bottoms, and suspended in fiberglass water trays. Each of the seven container sizes is replicated 50 times to allow meaningful statistical analysis. The containers were stocked with Stage IV *Homarus* in January 1975, but survivorship has been low due to heavy *Lagenothrix* infections. Disease treatment procedures have been developed and the containers will be restocked as larvae become available.

---

*This work was supported by several agencies. Research at the Encina and Scripps Institution laboratories was sponsored by the NOAA Office of Sea Grant, Department of Commerce, under Grant USDC 04-3-58-122. Work at the Redondo Beach laboratory and some aspects of research at the Scripps laboratory were sponsored by the Research and Development Program of the Southern California Edison Company under contracts U2603906 and U2384902.

DEVELOPMENT AND EVALUATION OF PROTOTYPE PRODUCTION MODULES FOR THE CULTURE OF *Homarus americanus* IN INDIVIDUAL REARING CONTAINERS.*

Jon C. Van Olst and James M. Carlberg

Department of Biology
San Diego State University
San Diego, California 92182

ABSTRACT

Sufficient progress has been made in most important areas of lobster culture so that we feel it is useful now to develop and evaluate models of the tanks, rearing containers, and other equipment that might be employed in commercial production. This is important because it is the only way in which meaningful assessments of related problems such as water treatment, food stability, and cost effectiveness can be made. Many different types of rearing systems have been devised, constructed and often discarded. Several systems are under evaluation in our laboratories and two appear particularly promising. These are the care-o-cell and the flush tray.

The care-o-cell system is essentially a shallow round tank based on the design of a primary sewage treatment clarifier in which floats a revolving group of screened-bottom rearing containers. The group of containers revolves beneath a radius arm from which jets of water discharge at an angle into each rearing compartment as it passes beneath . Models have been constructed in sizes 4, 5, and 10 feet in diameter. The group of rearing compartments is rotated by the force of the water input jets, in these prototype models; larger production units would be powered by gear motors. Our 10 ft diameter care-o-cell has a walkway spanning the tank for convenient access and feeding. In larger units the walkway could also house photocell-operated food delivery ports and harvesting systems.

---

*This work was supported by several agencies. Research at the Encina and Scripps Institution laboratories was sponsored by the NOAA Office of Sea Grant, Department of Commerce, under Grant USDC 04-3-58-122. Work at the Redondo Beach laboratory and some aspects of research at the Scripps laboratory were sponsored by the Research and Development Program of the Southern California Edison Company under contracts U2603906 and U2384902.

The flush tray system consists of a long, shallow rectangular tray divided into rearing compartments by vertical plastic partitions. Water supply and exit weirs are located along the two long sides of the tray, and the plastic partitions along this axis are perforated so that fresh seawater entering the system flows perpendicular to the long axis of the tray. The short plastic partitions placed cross-wise in the tray are not perforated, and are raised slightly off the bottom. A plastic toilet tank valve at the end of the tray is opened periodically and most of the water is flushed to waste. As this occurs the solid partitions act as dams so that the water is forced to flow rapidly across the bottom of the tank. This flow has a scouring effect and is quite effective in removing accumulated wastes. In this system, therefore, oxygenated water can flow slowly and evenly across the short axis of the tray while the waste flushing water is forced to move rapidly across the bottom of the tank.

Both of these rearing systems functioned adequately in our preliminary evaluations. Dye studies showed that water flow and waste removal patterns are effective. We are currently assessing problems of scale-up and various designs for the individual holding containers to be used in these systems. Waste removal efficiency is largely dependent upon the size, number, and shape of the perforations in the containers, and this aspect of the design also is under study.

A Los Angeles area company specializing in the vacuum forming of plastics has been contracted to fabricate trial containers and more than 60 prototypes have been made on wooden molds. Assuming that one or more of these designs proves satisfactory, metal molds will be constructed and the containers produced in larger quantity for further evaluation.

If either the care-o-cell or flush tray system ultimately proves satisfactory, we plan to construct full scale operational units with the cooperation and support of the Southern California Edison Company. The flush tray system could be conveniently supported on cantilever warehouse storage racks up to ten levels high. The chief advantage of this system is that it could provide very high production densities, which might be necessary if the proposed lobster farm is to be located on expensive coastal property where a highly intensive approach would be necessary.

058

Disease Problems Associated with Lobster Aquaculture

H.C. Schapiro and J.F. Steenbergen
San Diego State University
San Diego, California

One of the most severe problems facing the development of intensive culture of any species is the control of disease. To optimize productivity and economic feasibility, aquaculturists must use elevated temperature, maximum feeding rates, and minimum space per animal. Under these conditions, the animals will be under physiological stress, and thus be more susceptable to an increasing variety and frequency of diseases. Two diseases are now causing problems in mariculture efforts. Gaffkemia, a fatal bacteremia of lobsters, causes epizootics in the wild, in impoundments, and aquaculture facilities. Leucothrix, a filamentous bacterium, is associated with significant losses of larval and juvenile stages of cultured lobsters. In addition to these two diseases, we have isolated a number of gram negative bacteria from moribund lobsters provided to us by other West Coast Sea Grant investigators. The pathogenicity of these bacteria has not yet been assessed.

We have found a high incidence of the causative agent of gaffkemia on the exoskeleton of normal lobsters. These lobsters, although carrying the bacterium, are not internally infected, but could be a source of infection for other animals. Our studies have shown that both the California Spiny Lobster and the American Lobster can be immunized against gaffkemia. The immune system in invertebrates is primarily cellular. As a means of characterizing the cellular response, a system was developed for the short term maintenance of H. americanus hemocyte

suspensions. These *in vitro* studies are being correlated with protection of intact lobsters. Serological studies have demonstrated an antigenic determinant which is highly correlated with the virulence of a given strain. We are currently isolating and characterizing this "virulence" antigen.

Several problems involving filamentous bacteria have reached epizootic proportions. During the past year all of the major aquaculture groups have had difficulty rearing lobsters to the juvenile stages. This high mortality is presumably due to *Leucothrix* infections of the larval lobsters. Recently, a filamentous bacterium growing on gill rakers has been associated with a high mortality in juvenile lobsters. A similar problem has resulted in a virtual shutdown of some shrimp-rearing facilities.

We are studying the etiology of these presumed *Leucothrix* infections. This is being accomplished by preparing *Leucothrix*-specific fluorescent antibody reagent using pure cultures of *Leucothrix* from the American Type Culture Collection (ATCC). We also propose to isolate the presumed pathogens directly from lobsters and shrimp and test their pathogenicity. These isolates will be taxonomically identified and compared to the ATCC strains. We shall then study the *in vitro* susceptibility of the ATCC strains and the pathogenic strains to chemicals and antibiotics. Any potentially successful treatment will be tried *in vivo* at the affected aquaculture facilities.

DS7

Reproductive Cycles and Ovarian Development in *Homarus*

D.E. Aiken and E.H. Byard

Environment Canada
Biological Laboratory
St. Andrews, N.B.

The literature on *Homarus* contains numerous references to the occurrence of molting and egg laying in alternate years, and even some suggestion of a temporal relationship between mating and egg laying. It has also been suggested that the protracted period between successive spawnings can be routinely compressed by holding mature females in warm water. Our observations on the female reproductive cycle indicate that all of these concepts are only partially correct. For example, a majority of the 450 to 550 gram females from Northumberland Strait and Gulf of St. Lawrence routinely molt in August-September and lay eggs a month later. Different size lobsters from the same area may either molt, lay eggs, or do nothing. Observations on laboratory and field samples of this population over a 3-year period indicate each age-class has a characteristic pattern of molt and reproductive events. The immature female forms its secondary sexual characters over two successive molts, with ovarian vitellogenesis commencing during the summer of the second (puberty) molt. Ovarian maturation progresses through two distinct stages of vitellogenesis spanning 9-12 months. In nature this always includes a winter period, and a cold water exposure may actually be required at some point (as is the case in freshwater crayfish). First egg extrusion (Adult I) occurs approximately a year after the puberty molt, and the animal will molt again early the following summer (Adult II). The ovary commences its second cycle that summer as well, with the result that both the molt cycle and the ovarian cycle approach completion in the following (Adult III) summer. Proper synchronization at this point appears crucial, for if the ovary develops to the point where egg laying will occur either before or within three weeks after molt, the gravid ovary will be reabsorbed, which frequently happens when females of this size are pushed in warm water. As a natural breeding stock this age-class has the advantage of being cost-efficient producers of eggs for culture, and useful for synchronized planned matings with rapid egg production.

In the final month before egg laying, Female Specific Protein (FSP) can be detected by standard electrophoretic methods, and in some cases the blood becomes pale green. We have now developed a much more sensitive quantitative and qualitative assay using rabbit anti-lobster lipovitellin serum and immunochemical analysis to detect the presence of FSP in the blood. With this technique we hope to detect primary as well as secondary vitellogenesis and develop spawning prediction curves.

060

Induction and Inhibition of Molting in *Homarus*

D.E. Aiken and Susan Waddy

Environment Canada
Biological Laboratory
St. Andrews, N.B.
April 10, 1975

Lobsters of 65-80 mm carapace length display a circannian cycle of sensitivity to premolt induction, whether natural or induced, and this sensitivity is mediated by neurosecretory centers in the eyestalk. Natural premolt induction at 10 C occurs rapidly in spring, and slowly if at all in autumn. However, the difference between spring and autumn responses disappears when eyestalks are removed but can be reinstated by implantation of C_4 eyestalk tissue. Sensitivity to injected ecdysterone likewise varies seasonally, is mediated by eyestalk tissue, can be stabilized by eyestalk removal, and can be upset by eyestalk tissue implants. These results demonstrate the existence of an eyestalk factor analogous to crustacean MIH. Ecdysterone probably is not the inducer of premolt; its function is primarily that of mediating formation of new cuticle, and its target organ appears to be the epidermis. Ecdysterone doses sufficient to induce premolt are almost always fatal because new cuticle is formed and ecdysis occurs without completion of early proecdysial processes such as apolysis, gastrolith formation, limb buds, setagenesis, etc. Eyestalk removal followed by sequential ecdysterone injections permits successful completion of accelerated molt, suggesting strongly that a separate factor, controlled by the eyestalk complex, is responsible for induction of premolt and completion of the vital processes of early premolt. Temperature also influences sensitivity to exogenous ecdysterone. Dose levels of 0.5 and 1.0 µg/gbw are ineffective at 10 C but the higher dose becomes very potent when the temperature is increased to 17 C, demonstrating the necessity for standardized conditions in such experiments. Premolt induction can be inhibited by implantation of C_4 eyestalk tissue and by sequential doses of 0.5 µg/gbw FME, a juvenile hormone mimic. We have been unable to inhibit molting by surgical removal of suspected ecdysial glands, and recent results with insects suggest the role of ecdysial glands of both insects and crustaceans may be different than previously thought.

061

Lipid and Protein Requirements of *Homarus*

J.D. Castell and Jane F. Covey

Environment Canada
Halifax Laboratory
Halifax, N.S.
April 11, 1975

In lobster nutrition studies both physical and biochemical factors must be considered. Binders - either protein or polysaccharide - are necessary to prevent disintegration before food can be consumed. Even the best binders permit leaching of water soluble nutrients, and up to 60% of glucose may be lost after two hours in sea water. Leaching can be prevented by encapsulation, but some leaching may be necessary for the food to be attractive to lobsters. Solid fats such as hydrogenated coconut oil can be used to encapsulate the food pellet, but may themselves be detrimental to the health of lobsters.

Serum protein level, percent edible meat, vital organ size, tissue composition (lipid carbohydrate, protein and ash) and growth rate are used as condition indices in evaluating different diets. Adult lobsters require high levels of dietary protein; serum protein analysis showed that when diets containing 0, 20, 40, or 60% protein (as vitamin-free casein) are fed to adult lobsters, only the 60% protein diet permits maintenance of the serum protein level found in wild lobsters. This same result is substantiated by comparison of percent edible meat. We also determined the lipid requirement of lobsters by feeding diets containing either corn oil (linoleic), cod liver oil (linolenic), or hydrogenated coconut oil (deficient in essential fatty acids). These experiments revealed a requirement for Omega 3 (linolenic) fatty acid. Additional experiments with sterol-free and sterol-added (cholesterol) cod liver oil revealed that lobsters also require a dietary source of sterol. In juvenile lobsters we found the optimum cholesterol level to be 0.5% of the diet dry weight. Ongoing experiments include effect of -Carotene, mineral mix, glucosamine, and vitamins A, D, E, and K on juvenile lobsters.

062

Quantitative Lobster Genetics and Culture Applications

Glen S. Jamieson

Applied Marine Research Limited
5112 Prince St.
Halifax, N.S.

Knowing the heritabilities of quantitative characters important to lobster culture permits a prediction to be made as to the likelihood of success and the time required to develop a true-breeding strain possessing desired characteristics. How well an individual's offspring will inherit the characteristics which made the parent desirable is determined.

In initial studies, the heritabilities of the tolerances of newly-hatched inshore and offshore lobster larvae to low salinities and sodium hypochlorite concentration (bleach) were determined. No difference in tolerance to low salinities were observed, but inshore lobster larvae were found to be more tolerant to sodium hypochlorite than were offshore lobster larvae. Additive heritabilities for these tolerances did not differ between the two populations, and for both characters averaged $0.41 \pm .20$.

The genetic characteristics of lobster populations should now be investigated to permit evaluation of the within and between population variance as only in this way can a planned programme of lobster strain development be designed.

063

Initial Development of Artificial Diets for the Lobster, Homarus americanus*

Douglas E. Conklin, Kathryn Devers, and Robert A. Shleser

ABSTRACT

Growth trials are described for a series of artificial diets designed for the lobster, Homarus americanus. Methods of preparation and ingredients used for each of the manufactured feeds are presented. Details of experimental conditions are provided and results of growth trials are examined from several standpoints. The influence of animals of differing genetic backgrounds on the results are also delineated. Experimental standards which would facilitate comparison of nutritional data between investigators are proposed.

*This work was supported in part by Grant #94-3-158-#A4 from the Sea Grant Agency, NOAA, U. S. Department of Commerce, and conducted at the Bodega Marine Laboratory of the University of California.

064

FORMULATIONS OF RATIONS FOR THE AMERICAN LOBSTER, HOMARUS AMERICANUS*

by

Robert Shleser[1] and Margie Gallagher[2]

A program has been developed for compounding and evaluating artificial diets for the lobster, Homarus americanus. Feeding trials using brine shrimp have produced growth rates that exceed those described in feeding trials where clams and green crab were fed. (Brine shrimp are expensive and not routinely available.) The growth rate on brine shrimp, however, can be used as a standard in evaluating new diets which have been formulated from economical sources of the dietary components. Commercially available diet formulations have been compared which were developed in several aquaculture laboratories. The studies conducted show that diet affects both growth components; molting frequency and size increment per molt. This study suggests that future emphasis be placed on matrix-type constituent analysis of brine shrimp in comparison to other diets in order to determine which elements or rations of elements are essential dietary components of the lobster. Our progress in this area will be discussed.

---

[1]Robert Shleser - Asst. Prof., Aquaculture Lab., Dept. of Food Science and Technology, University of California, Davis.

[2]Margie Gallagher - Graduate Student, Dept. of Food Science and Technology, University of California, Davis.

*This work was supported in part by Grant #04-3-158-22-FA4 from the Sea Grant Agency, NOAA, U.S. Department of Commerce, and conducted at the Bodega Marine Laboratory of the University of California.

065

STUDIES OF THE EFFECTS OF FEEDING FREQUENCY AND SPACE ON THE GROWTH OF THE AMERICAN LOBSTER, HOMARUS AMERICANUS

By

Robert A. Shleser[1]

Because the lobster is cannibalistic, we believe that lobsters which have been grown beyond the juvenile stages must be separated and held individually. As a result, it is essential to determine the effects of habitat configuration, spacial limitation on the growth rate of the individually held lobsters.

As a first step in determining the optimum configuration for holding lobster, rectangular, square and circular environments have been examined. Results show that configuration has no significant effect on growth. However, growth is limited by the size of the habitat before available space is filled. Experimental results on the relationship of spacial requirements to growth will be presented.

Lobsters which are held individually must be individually fed. In order to obtain the optimal growth rate at the lowest cost, we have examined the effect of feeding lobsters an amount of food which exceeds the daily demand. Food was given at one, two and three day intervals and growth rate was measured. The results of these studies indicated that at least daily feeding was necessary for juvenile lobsters. The implications of these studies will be discussed. A preliminary examination of the growth of male and female lobsters under controlled conditions revealed a more rapid growth of male lobsters under the same conditions.

---

[1]Robert A. Shleser, Asst. Prof., Dept. of Food Science & Technology, University of California, Davis. This work was supported in part by Grant #04-3-158-22 FA4 Sea Grant NOAA and was conducted at the Bodega Marine Laboratory of the University of California.

Preliminary Acute Toxicity Studies of Some Inorganic Compounds on *Homarus americanus*

Norman Gravitz, Linda Gleye, George Tchobanoglous and Robert Skinner

ABSTRACT

The acute toxicity of ammonia, and nitrite and nitrate anions on *Homarus americanus* the Maine Lobster, are reported. The ammonia concentrations at which 50% mortality occurs within 96 hours (LC_{50} 96 hours) is 1.2 ± 0.1 and 1.4 ± 0.1 mg N for one and three gram animals respectively. Exposure to ammonia concentrations of five fold lower than the acute lethal levels sensitizes the animals to later ammonia exposure. A progressive necrosis of the hepatopancreas is observed. Concentrations of up to 500 mg NO_3^- - N/l and 100 mg NO_2^- - N/l are not lethal in 96 hours, and no gross behavioral or physical effects are noted.

0 16

Culture of Lobsters in "Green Water"

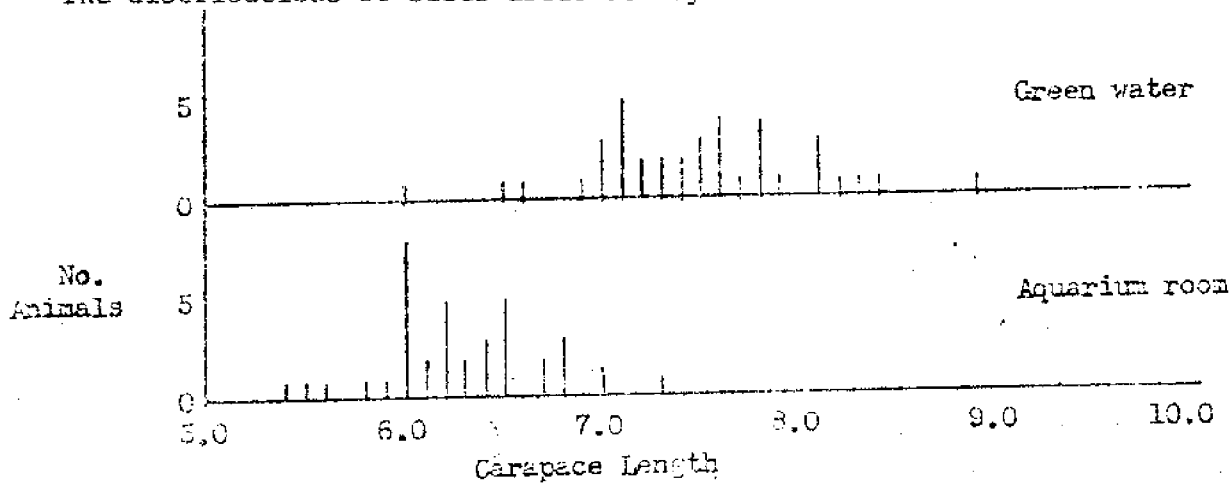
M. Hartman and L. Goesford

Bodega Marine Laboratory
Bodega Bay, Ca. 94923

Preliminary Evaluation. A mass-rearing technique for juvenile lobsters has been completed. A semi controlled biological system in which select primary producers, (phytoplankton), 1° consumers, (brine shrimp) and 2° consumers (lobsters) are brought together in one system under near ambient conditions was tried.

For the preliminary evaluation of green water culture, 44 stage 4 juvenile lobsters (ave. size 4.5 mm) were put into a single tank. The tank was inoculated with Carteria sp., 3 parts, Dunaliella primolecta, 2 parts, and Phaeodactylum tricornutum, 1 part, to a cell density of 5 x 10^4. Adult brine shrimp (Artemia salina) were added to a concentration of ~1/100ml. The tank was in a green house structure open at the surface. Water was agitated by air bubbling from a perforated pvc tube along one wall and single tubes in the opposite corners. The water was not nutrified (although there was some carry over from the inoculation). The water was changed in its entirety about every 10 days to keep desireable species of phytoplankton as main primary producers. After 2 exchanges the phytoplankton inoculum was limited to Carteria sp. (2 parts) and Dunaliella primolecta (1 part) as Phaeodactylum overgrew to 1 x 10^5 cells/ml.

The distributions of sizes after 90 days were as follows.



The average carapace lengths for green water and the aquarium system are 7.4 mm and 6.3 mm respectively. The mortality in green water was 13.6% whereas estimated mortality in the aquarium system is 40 to 50%.

Economic Considerations. The economic comparison of green water culture versus clear water based upon these preliminary data must of necessity be crude. The most significant factors in favor of green water are the faster growth rate and higher survival. The growth rate was a factor of 3/2 faster and survival was approximately 3/2 higher for green water.

068

BIOCHEMICAL GENETICS OF LOBSTERS (HOMARUS). I. GENETIC VARIATION AND THE STRUCTURE OF AMERICAN LOBSTER POPULATIONS[1]

Martin L. Tracey[2], Keith Nelson, Dennis Hedgecock,
Robert A. Shleser, and Mary-Lou Pressick

University of California
Bodega Marine Laboratory
P.O. Box 247
Bodega Bay, California 94923

Abstract

Six populations of lobsters, Homarus americanus Milne-Edwards, were surveyed for genetic variation at 43 loci encoding electrophoretically detectable proteins. Rather low levels of genetic variability were found, the average proportion of heterozygous loci per individual being 3.8%. Genetic variation is concentrated at only 8 loci, with just three loci -- Acph-1, Est-2, and Tr-2 -- having proportions of heterozygotes greater than 20%. Interpopulation differences are small; the genetic identity averaged over all loci, I, is above 0.99 in all but two comparisons. Differentiation between populations was found only at the Me locus, but the degree of this differentiation supports the suggestion from previous migration and morphological studies that H.americanus is subdivided into a number of more or less geographically isolated inshore and offshore populations. These local populations are none the less genetically very similar.

1. Research supported by Grant #04-3-158-22-FA4 from the Sea Grant Agency.

2. Present address: Department of Biological Sciences, Brock University, St. Catharines, Ontario, Canada L2S 3A1.

069

INTRODUCTION

The amount of genetic variation within and between populations of the lobster Homarus is of considerable practical and theoretical interest. On the one hand, management of natural lobster populations requires sound knowledge of population structure. Likewise, genetic improvement of cultured lobsters, either through selective breeding or hybridization, depends directly on hereditary differences between individuals and populations. Application of modern biochemical genetic methodology to practical problems of lobster biology produces information important to basic research in the fields of population and evolutionary genetics.

Gel electrophoresis of tissue extracts followed by selective staining of various enzymes and proteins is a technique whereby genetic differences between individuals from the same population or from different populations or species can be demonstrated. Eighteen functionally different enzymes and proteins coded by forty-four genes have been studied in hundreds of lobsters. This survey has produced estimates of genetic variability within typical lobster populations, between geographically separate populations of the American lobster, and between the two species, H. americanus and H. gammarus. Furthermore, classical genetic inheritance has been confirmed so far for five of the eight most variable enzymes in these populations. Our results and their implications may be summarized in three parts:

M.L. Tracey, R. Hudson, D. Ridgecock and R. Shleser
Bodega Bay Marine Laboratory
Bodega Bay, Ca. 94923

1) Population Structure of Homarus americanus

In general, our survey revealed that relative to other invertebrates the typical lobster population has only a moderate level of genetic variability. The ten most frequent genotypes (in our analysis each genotype is represented by forty-four individual loci or genes) comprise over eighty percent of the individuals in a population. The biological significance of such biochemical variation in animal populations is a much debated problem in population genetics. We are presently contributing to this basic research by gathering data on other decapod crustaceans that have commercial importance as well.

As in most animal species geographically separate lobster populations are genetically very similar. At each of forty-three enzyme loci one or two alleles have similar frequencies from the Gulf of St. Lawrence to the Hudson Canyon. (Eight populations sampled were located near Prince Edward Island; Yarmouth, Bay of Fundy; Damariscove Island, Maine; Wood's Hole and Martha's Vineyard, Massachusetts; and the offshore canyons Hudson, Lyndononia and Hydrographer.) The finding of geographic uniformity at these loci reveals nothing of population structure, but an additional gene, the Malic enzyme locus (Me), is an exception to the rule. Two Me alleles are electrophoretically detectable. The Prince Edward Island population consists almost entirely of individuals homozygous for the Me fast allele (fast phenotypes), while the Massachusetts inshore populations consist entirely of individuals homozygous for the Me slow allele (slow phenotype). Offshore populations have these Me alleles in equal frequencies, the ratio of the three phenotypes in these populations being 1 slow: 2 slow-fast: 1 fast. Because genetic differences

cannot be maintained in the face of reproductive exchange between populations except with high differential selection pressure, it is very likely that lobster populations are somehow isolated from one another. In combination with data on differential migratory patterns in inshore and offshore populations, our genetic data make it unlikely that lobsters from various inshore and offshore localities form a single population.

2) Allozyme Tagging

After surveying natural populations for biochemical variation, we collected berried females in order to study the inheritance of enzyme variants among their offspring. We did indeed confirm that five enzymes are each encoded by single independent genes. More importantly, we paved the way to realizing the potential of allozymes (allelic enzyme variants) as markers in laboratory and field experiments.

Allozymes are an ideal solution to the problem of tagging crustaceans since the "tag" is the actual tissue of the animal which cannot be shed with the molt. "Tags" are detected by electrophoresis of tissue from single walking legs removed from individual lobsters without sacrifice. The principle of allozyme tagging is based on consideration of the probability of occurrence of individual genotypes. For example, among the progeny of one female was a juvenile with a fast phosphoglucose isomerase-3 genotype ($\underline{Pgi\text{-}3}^{105/105}$), a slow-fast Pgi-4 genotype ($\underline{Pgi\text{-}4}^{98/100}$), and a slow-fast phosphoglucomutase -1 genotype ($\underline{Pgm\text{-}1}^{100/103}$). Separately, these genotypes occur in the following percentages of the typical population: $\underline{Pgi\text{-}3}^{105/105}$ @ .02%, $\underline{Pgi\text{-}4}^{93/100}$ @ 26% and $\underline{Pgm\text{-}1}^{100/103}$ @ 39%. The probability of these

three genotypes occurring in the same individual, however, is the product of their separate probabilities, or two in one hundred thousand!

With recent improvements in lobster breeding and hatching it is feasible that large cohorts having otherwise rare combinations of genes can be synthesized and raised to fourth or fifth stage. Release of these marked lobsters at selected study sites and the subsequent monitoring of their survival and dispersion will provide for the first time data on the efficacy of restocking practices.

3) Differences Between American and European Lobsters

We have recently studied thirty-five loci in twenty H. gammarus from the Bay of Caernarvon in the Irish Sea. Qualitative differences between these animals and our previous samples of H. americanus occur at four loci and quantitative differences in allelic frequencies are present at an additional three loci. The remaining twenty-eight loci had identical genotypic distributions in both species. Over all loci, the average genetic similarity between the species of Homarus is .85 compared to a genetic similarity among H. americanus populations of .99.

Because of the genetic divergence of these species, interspecific hybrids are expected to be heterozygous at one out of five loci instead of an expected heterozygosity in the parental species of one out of twenty loci. Animal and plant breeders have often demonstrated that increased heterozygosity may lead to increased performance or yield. A fourfold increase in heterozygosity in American x European hybrids may have aquaculturally desirable results. Hybridization studies with lobsters are, therefore, of considerable significance.

Disease Situations of Homarus americanus

070

W.S. Fisher, E.H. Nilson, L.F. Pollett and R.A. Shleser
Bodega Marine Laboratory
Bodega Bay, Ca. 94923

Microbiology/Pathology

It is becoming increasingly apparent that more emphasis is needed in larval rearing techniques. The present methods were sufficient to produce post-larval animals with great success just a few years ago. Recently, however, there have been a number of difficulties in both flow-through and warm water rearing. The difficulties have been generally described as disease situations. As such, they are dealt with on the level of disease control, i.e., identification of disease organism, use of chemotherapeutics or antibiotics, cleansing of systems, etc. We feel that it is equally as important to reconsider and evaluate optimal rearing parameters.

Some of the diseases which have been responsible for losses in lobster rearing systems are described below:

Filamentous contamination:

Filamentous micro-organisms are found to infest the external surfaces of both eggs and larvae. Mortalities are felt to be due to anoxia when fouling occurs on the egg membrane and larval gills or to restriction of molting when fouling occurs on larval exoskeleton. The bacteria involved are similar to Leucothrix mucor, to Beggiatoa, and to Vitriocella. The filamentous cyanophytes encountered resemble Oscillatoria and Rivilaracea. Vorticellid protozoans are often present and aid the filamentous micro-organisms in trapping diatoms and detritus to further contaminate the surface. These organisms rely on holdfasts for stability and do not penetrate the egg membrane or larval exoskeleton. It is presumed that their major nutrient source is from the surrounding seawater and that their growth is effected by water quality.

References: 1, 2, 3.

Lagenidium sp.

This phycomycecetous fungus is capable of killing an entire population of larva in a system within a few days. The mycelium invades and completely destroys the tissues of the animal, leaving only the exoskeleton. It has also been observed in lobster eggs and results in discoloration and necrosis.

The fungus is similar to the Lagenidium that infects crab and shrimp, but may be a different species. The disease is most destructive when the antibiotic streptomycin is used to rid larva of filamentous bacterial infestations. This treatment apparently reduces the microbial competition for infestation of the larva.

References: 2, 3, 6.

Undescribed Dual Bacterial Infection

Preliminary observations indicate that a bacterial organism with chitinolytic properties followed by another bacterial organism resembling Cytophaga are responsible for mortalities in larval and early post-larval stages. The primary invader has been isolated on chitin media and shows chitinolytic properties. The secondary invader is observed in tissues near the site of primary infection. The overall appearance is an orange or yellow area on the animal, usually on the ventral abdomen.

No references

Halipthoros milfordensis

H. milfordensis is easily recognized on post-larval lobsters. The phycomycete is slightly chitinolytic and causes melanization at the site of infection. In very young post-larva the disease may occur anywhere on the animal, however, larger juveniles with thicker exoskeletons are predominately infected in the thin chitin membrane of the branchial cavity. Diagnosis often requires lifting the carapace from the gill area. H. milfordensis causes tissue destruction and has been observed to interfere with ecdysis when host melanization binds the shedding exoskeleton to the newly forming exoskeleton. Fortunately, losses are not heavy if moderate cleaning procedures are maintained.

References: 5.

Various systems and treatments have been used to overcome the diseases found in the larval rearing systems. The greatest success was achieved in an ultraviolet irradiated semi-closed seawater system with a periodic dipping

of eggs and larvae in malachite green (Reference 3). This method provided good yields of post-larval lobsters in spite of observed infestations of Lagenidium and filamentous micro-organisms. Eggs were dipped in a 5 ppm solution of malachite green for 10 minutes prior to entering the hatch tanks and larva were dipped 2 minutes in the same solution 2-3 times weekly until reaching the 4th stage.

Although this method was productive for a particular type of disease situation, it may not be sufficient for all. With this in mind it has been necessary to establish toxicity levels for the use of malachite green on lobster larva. Using the semi-daily malachite green dipping technique described earlier, it has been found (unpublished data) that larvae can easily survive an 8 minute dip in a 20 ppm concentration of malachite green. Similarly, they are successful at a maximum of 4 minutes in a 40 ppm malachite green dip.

The necessity of treating lobster larva for successful production underscores the ineffectiveness of present larval rearing methods (Reference 4). Since the diseases encountered are numerous and the agents involved are a variety of micro-organisms, it is unlikely that a single treatment regime will be sufficient. In addition to disease control measures it is becoming obvious that a better understanding of the physical parameters of the system is needed. The animals are undoubtedly under various forms of stress that make them vulnerable to the various diseases. Since many of the diseases are found on eggs as well as larva, pre-hatching treatment of ovigerous females should also be investigated. It is very important at this time to optimize the density, feeding, lighting, water quality, temperature, flow rates and other parameters for the egg and larval stages of lobster.

Publications

1. Nilson, E.H., Fisher, W.S. and Shleser, R.A. 1975. "Filamentous infestations observed in eggs and larvae of crustaceans". Submitted to the Proceedings of the World Mariculture Society.

2. Fisher, W.S., Nilson, E.H. and Shleser, R.A. 1975. "Diagnostic procedures for diseases found in egg, larval and juvenile cultured lobsters". Submitted to the Proceedings of the World Mariculture Society.

3. Fisher, W.S., Nilson, E.H. and Shleser, R.A. 1975. "Hatching and rearing of lobster larvae in a disease situation". Aquaculture. In press.

4. Shleser, R.A., Van Olst, J., Ford, R., Schuur, A., Hughes, J. and Fisher, W.S. "Methods for hatchery production of H. americanus juveniles". Manuscript in preparation.

5. Fisher, W.S., Nilson, E.H., and Shleser, R.A. 1975. Effect of the fungus Haliphthoros milfordensis on the juvenile stages of the American lobster, Homarus americanus". Journal of Invertebrate Pathology. In press (May/June issue).

6. Nilson, E.H., Fisher, W.S. and Shleser, R.A. 1975. "A new mycosis of larval lobster". Submitted to the Journal of Invertebrate Pathology.

071

by

Louis W. Botsford[1], H. E. Frauch[2] and Robert Shleser[3]

ABSTRACT

Engineering methods of system analysis and optimal control theory are applied to the commercial raising of aquatic species. Mathematical models of aquaculture system components are developed and optimization theory is applied to the design and operation of an aquaculture facility. The models are formulated in terms of variables which describe the condition of the system (State Variables) and variables which influence the condition of the system (Control Variables). The State Variables are the number of animals in a batch, their average weight, and their cost. The Control Variables relate to the water temperature, water recirculation or replacement, space per animal, amount of food per animal, and kind of food. Differential equations which relate the State Variables to the Control Variables are presented. Mathematical optimization techniques are used to determine the values of control variables which minimize the cost. These techniques are applied to the operation of an aquaculture facility to farm lobsters. Sample numerical results are presented and the interaction of these theoretical results with practical considerations are discussed.

---

[1]Botsford is the systems engineer for the Aquaculture Group in the Department of Food Science and Technology at the University of California, Davis (Sea Grant #FA4).

[2]Frauch is a staff scientist with the Lockheed Palo Alto Research Laboratory.

[3]Shleser is an Assistant Professor with the Department of Food Science and Technology at the University of California, Davis.

[4]This work was supported in part by Grant # from the Sea Grant Agency and was conducted at the Bodega Marine Laboratory of the University of California.

072

AN ECONOMICALLY OPTIMUM AQUACULTURE FACILITY[3]

Louis W. Botsford[1], H.E. Rauch[2], A.H. Schuur[1] and Robert Shleser[1]

ABSTRACT

A method for determination of optimal values of control variables in an aquaculture facility has been presented previously (Botsford, Rauch, and Shleser, 1974). This paper describes major improvements in the optimization approach and use of the approach in design and evaluation of real culture systems. Added features of the optimization approach include determination of optimum market size, discrete rather than continuous space allotment, and a new waste treatment model. In addition, recent experimental results and proposed physical system designs have been incorporated in the model enabling more accurate optimization and more realistic predictions. The influence of geographic location, changes in cost of various components, and experimental uncertainty in biological parameters on optimal values of control variables and final costs are described.

[1]Botsford, Schuur, and Shleser are with the Aquaculture Group in the Department of Food Science and Technology at the University of California, Davis.

[2]Rauch is a staff scientist with the Lockheed Palo Alto Research Laboratory.

[3]This work was supported in part by Grant # PA4 from the Sea Grant Agency and was conducted at the Bodega Marine Laboratory of the University of California.

073

A Polyculture Approach to Rearing Lobsters

Joan R. Mitchell
Environmental Systems Laboratory
Woods Hole Oceanographic Institution, Woods Hole, MA. 02543

Lobster fry were hatched and maintained in the laboratory through early post-larval stages(6.5 to 14 mm Carapace length). These animals were then introduced into cement raceways (12x 1.2 or 2.4 x 1.5 m deep), which contained stacked trays of oysters. After seven months (September 1974 to March 1975) residence in the raceways, the animals were removed. The results show that the lobsters grown in the two heated raceways(approximately 15 °C) showed an average increase in size from 8.96 to 25,85mm carapace length, with several individuals exceeding 40.00mm carapace length. The total weight of these animals at introduction was 138.7 g, and increased to 2195.3 g during the seven month period. Thus, the mean weight increase/ individual was from .35 to 17.70 g. A total of 390 animals were introduced into these two raceways at a mean stocking density of 11.8 individuals / m^2;the survival rate was 32%.

Animals grown in ambient water (0 to 20°C) increased in size from 8.66 to 12.27 mm mean carapace length, with a total weight increase of 18.98 to 38.00 g, or a mean weight increase/ individual from .26 to 1.19 g. Seventy three animals were introduced into this raceway at a stocking density of 7.77 individuals/ m^2, with 44% of the animals surviving.

Twelve animals were held in individual containers in a heated raceway for the same period of time. The mean increase in carapace length was 13.04 to 18.25 mm, with a total weight increase of 14.85 to 52.70 g (mean increase / individual: 1.24 to 4.40 g). These animals showed 100% survival at a stocking density of 92.3 individuals/ m^2.

In all cases the animals existed on naturally occurring food species, i.e. amphipods isopods, polychaete worms, etc., which grew abundantly in the raceways. No supplemental food was added during the entire seven month period.

074

Delay of Molt in Juvenile Lobsters

J. Stanley Cobb and George R. Tamm
Department of Zoology
University of Rhode Island

Previously reported evidence (Cobb and Tamm 1974, and in press) has shown that when juvenile lobsters are held in pairs, the subordinate lobster will delay molt by as much as 100% of the normal intermolt period. Experiments in which the lobsters are physically separated but allowed chemical or visual communication across a barrier showed no delay in molting. We conclude that lobsters must have physical contact to produce the observed delay. In system design terms, this means that lobsters must be separated for maximum molting rate. When lobsters are completely isolated, they spend more time in simulated shelter than when in contact either chemically or visually. When in contact both chemically and visually the time is even less. This is interpreted to mean the more information concerning the presence of another lobster there is, the more active the lobster will be.

Throughout our experiments we have consistently seen trends (never statistically significant) indicating that when lobsters are held in chemical communication, molting rate and synchrony are facilitated. We do not know whether this is a real phenomenon; however, further research is strongly suggested.

Molting patterns exhibit one other temporal clustering. In fifth and sixth stage lobsters, a diel rhythm in ecdysis is apparent, with a unimodal peak several hours after the onset of light.

075

Behavioral Bioassays in Lobsters

J. Stanley Cobb and George R. Tamm
Department of Zoology
University of Rhode Island

Several aspects of the behavior of lobsters in a closed, unfiltered water system were monitored and compared to that in an open system. In the closed system ammonia concentration increased from .02 ppm to 22.5 ppm over a period of 31 days then decreased rapidly. Ammonia concentrations in the open system remained very low. Feeding behavior (time to alert, time to grasp food and amount eaten) showed no difference between the two systems until very late in the experiment. Burrowing behavior also was unchanged.

A sequential and temporal analysis of burrowing behavior has been developed from the data obtained in this experiment. Burrowing behavior consists of six behavioral units which, in order of relative frequency of occurrence, are: Rake, Bulldoze, Backwards Dig, Burrow Turn, Tail Carry, and Pleopod Fan. Specific pathways -- certain units following others -- could be identified. Changes in number and duration of the units during a 15-minute burrowing period can be correlated with the progress of burrow construction.

c 76

Agonistic Behavior of Juvenile Lobsters

J. Stanley Cobb and George R. Tomm
Department of Zoology
University of Rhode Island

Interaction between paired lobsters results in the formation of a dominance-subordinance ranking, and it is always the subordinate lobster that delays molting. At or just before the time of molt, the dominance relationship reverses. The dominant (molting) animal becomes subordinate to the previously subordinate lobster. Ecdysis is preceded by a short period of intense agonistic behavior in which the lobster about to molt is very aggressive. Then as the old exoskeleton starts to lift, the molting animal retreats to its shelter. Any agonistic activity between this time and time of ecdysis may result in dominance reversal. After molting, which occurs in the shelter, the cast exoskeleton is pushed out of the shelter and very frequently the non-molting lobster comes to the shelter and starts to eat the exoskeleton. The cast may act as a decoy, distracting the second lobster from its newly molted (and vulnerable) partner. The changes in agonistic behavior over the molt cycle and particularly at the time of ecdysis imply hormonal regulation of aggressiveness. Experiments along these lines are planned.

When lobsters are held in groups larger than two, the evidence for the molt delay phenomenon is not clear. Others have shown the density of lobsters and the density of shelters to be important influences on growth rate and survival. When shelter density is varied, the spacing between shelters generally is also. When lobster density and shelter density are held constant but space between shelters is varied, molt rate is not affected, but survival is lowest when shelters are close together and highest when they are at maximal distance. Aggressive behavior is most intense when shelters are closely spaced but least intense when they are far apart.

077

Some Factors Influencing the Outcome of Shelter Competition in Lobsters (*Homarus americanus*)

David J. O'Neill and J. Stanley Cobb
Department of Zoology
University of Rhode Island

Two hundred fifty competitions for a single shelter were held between selected pairs of lobsters. Large lobsters won over small lobsters; male lobsters over females; intermolt lobsters over premolt and postmolt lobsters; lobsters with one or two claws over lobsters with fewer claws, and lobsters with claws one cm longer than their opponents' over lobsters with smaller claws. Prior residence in the shelter, right or left position of the crusher claw, or absence of both antennae did not influence the outcome of shelter competition. Dominance was the controlling factor in capturing or holding a shelter. Differences in the number of opponents' claws increased frequency of shelter sharing. Absence of both competitor's claws resulted in shelter sharing 90% of the time. Field observations indicated that laboratory stresses may be responsible for the clear-cut relationship between dominance and shelter possession.

078

AN EXPERIMENTAL AQUACULTURE SYSTEM FOR AMERICAN LOBSTER, HOMARUS AMERICANUS

A. N. Sastry
Graduate School of Oceanography
University of Rhode Island
Kingston, Rhode Island 02881

The technical feasibility of lobster culture from eggs to about market size animals has been demonstrated in a recirculating sea water system. Data has been gathered on molt frequency, synchrony, weight gain and survival of lobsters grown for over a 30 month period. Comparative data on growth characteristics of lobster juveniles has also been obtained for selected fast-growing animals and for eyestalkless animals. Experiments on growth were also conducted with animals maintained in flow through heated and flow through ambient sea water. Water quality changes have been monitored at intervals throughout the grow out period for all the culture systems. Experiments currently planned on growth and survival of communally held animals in the sea water systems will be compared with the base line data obtained for individually maintained animals under similar conditions.

079

EFFECTS OF SPACE ON GROWTH AND SURVIVAL OF LOBSTERS, HOMARUS AMERICANUS

A. N. Sastry, Doug Wilcox, and John Laczak
Graduate School of Oceanography
University of Rhode Island
Kingston, Rhode Island 02881

Under intensive culture conditions, the space requirements for juvenile lobsters growing to a market size is an important economic consideration. Studies have been conducted to determine the minimum space required for maximum growth of lobsters from the first juvenile stage to 14th juvenile stage (200 g.) in a recirculating sea water system. Available space sizes investigated have no effect on the growth rate of the early juvenile (5-7) stages. In the later stages, the growth rate of the animals in the smaller spaces was less than that of those in the larger spaces. The growth rate of these later stages is directly related to the space sizes. These results on the differences in growth rate of animals maintained in different space sizes are being compared with growth rates of field animals and animals grown under laboratory conditions.

080

THE EXCRETION OF AMMONIA BY LARVAL AND JUVENILE STAGES OF AMERICAN LOBSTER, HOMARUS AMERICANUS

A. N. Sastry and Shirley E. Barton
Graduate School of Oceanography
University of Rhode Island
Kingston, Rhode Island 02881

The ammonia excretion rates for all the larval and juvenile stage lobsters to the market size have been determined. The rate of ammonia excretion was higher for the larval stages than the early juvenile stages. The rate of excretion remained at a constant level (17 ug atoms/g/day) in the juveniles following the fourth juvenile stage to the market size animals. The intermolt animals have a higher rate of ammonia excretion than the premolt animals in all the stages examined. The rate of ammonia excretion was increased immediately after feeding the animals and decreased to a level characteristic for the unfed animals after four hours.

081

THE AMMONIA TOLERANCE OF LOBSTERS, HOMARUS AMERICANUS

A. N. Sastry and John Laczak
Graduate School of Oceanography
University of Rhode Island
Kingston, Rhode Island 02881

Ammonia is extremely toxic to culture organisms. Sublethal levels of ammonia in culture sea water may inhibit growth and also increase the susceptibility of the organisms to other unfavorable conditions of the culture system. Therefore, the information on the ammonia tolerance limits of different life cycle stages and long-term chronic effects on growth and survival are valuable for management of the water quality in the culture system. The toxicity of undissociated NH_4OH to larval and juvenile stage lobsters has been determined for 24 and 48 hours. Tolerance increases with development, larval stages being the most sensitive, the LD_{50} concentrations ranging from 3.5 mg/l NH_4OH for the 1^{st} stage larvae to 10 mg/l NH_4OH for the 4^{th} stage larvae. Juvenile stages have much higher tolerance levels, ranging from approximately 25 mg/l for 1^{st} stage juveniles to 250 mg/l for the 12^{th} and 13^{th} stage juveniles. Experiments determining the long term chronic effects of sublethal concentrations of ammonia on growth, molting and survival are currently in progress.

012

FACTORS AFFECTING SURVIVAL OF COMMUNALLY REARED LARVAL AND JUVENILE LOBSTERS, HOMARUS AMERICANUS

A. N. Sastry and Lynne Zeitlin-Hale
Graduate School of Oceanography
University of Rhode Island
Kingston, Rhode Island 02881

Larval and early juvenile stage lobsters, Homarus americanus, were maintained communally and individually to determine the effects of group interactions on survival. Survival of individually held animals was higher in all life cycle stages examined. Among communally held lobsters, survival was lowest for second stage larvae (30%), then progressively increased in the later larval and first juvenile stage to 68%. Mortalities among communally held lobsters were observed primarily during each group's molt period. Molt synchrony within each group was positively correlated with survival. A similar relationship between molting and mortality was observed in groups of freshly hatched larvae maintained to the early juvenile stages when population density was held constant. If some of the many behavioral and physiological factors leading to high mortalities during a group's molt period were controlled, communal rearing would be a viable possibility for commercial lobster aquaculture.

(Abstract of paper submitted to Marine Biol. for publication, 1975.)

083

THE EFFECTS OF ENVIRONMENTAL MANIPULATION ON LOCOMOTOR ACTIVITY AND AGGRESSIVE BEHAVIOR OF JUVENILE LOBSTERS, HOMARUS AMERICANUS

Lynne Zeitlin-Hale
Graduate School of Oceanography
University of Rhode Island
Kingston, Rhode Island 02881

The effects of environmental manipulation on the locomotor activity patterns, aggressive levels, and certain agonistic behaviors of juvenile (IX-XIII stage) American lobsters, Homarus americanus (Milne-Edwards) were investigated. The following variables were tested in a set of factorial experiments: lobsters individually or communally maintained, shelters present or absent, seawater flow through or recirculating. These particular parameters were chosen both for their importance to the behavioral ecology of juvenile lobsters and their potential importance in lobster aquaculture systems.

Activity was recorded by a mechano-electrical device for 5-9 day experimental periods with a 12:12 light:dark cycle. All lobsters maintained individually had similar levels of activity. When the same animals were communally maintained, there appeared to be social inhibition of activity; however, communally held lobsters without shelter were twice as active as those provided with shelter. The activity levels of groups of communally held lobsters decreased over the experimental period, but those groups with shelter had more uniform decreases.

All lobsters became somewhat entrained to the light:dark cycle and were nocturnally active. The degree of synchrony of activity was, however, strongly

influenced ($p < .001$) by shelter and other lobsters. Individually maintained lobsters all showed good entrainment, although those provided with shelter were slightly more synchronous. The presence of conspecifics desynchronized activity patterns when shelter was not provided.

Aggressive level, as measured by the number of agonistic encounters per observation period, decreased with time for groups of three lobsters in all experimental environments, but the temporal patterns were different for communally held lobsters in flow through and recirculating seawater. Preliminary observations on 12 stereotyped agonistic behaviors also suggested differences between these groups. The decrease in aggressive level primarily resulted from inhibition of activity, probably caused by dominance hierarchy formation. When two lobsters met, the probability of an agonistic encounter remained about 0.8 throughout the experimental period; however, decreasing activity levels resulted in progressively fewer encounters.

The results suggest several approaches for limiting aggression among communally maintained lobsters in aquaculture systems by environmental manipulation.

(M.S. Thesis in preparation.)

084

MOLT ACCELERATION BY EYESTALK ABLATION OF LARVAL AND JUVENILE LOBSTERS, HOMARUS AMERICANUS

A. N. Sastry and John Laczak
Graduate School of Oceanography
University of Rhode Island
Kingston, Rhode Island 02881

In crustacea, ablation of eyestalks generally accelerates molting. This has also been demonstrated to occur in the larger size juvenile lobsters. In application of this technique for aquacultural purposes, it is necessary to demonstrate not only molt acceleration, but also acceptable survival of the animals with corresponding increase in weight gain. Experiments have been conducted beginning with first stage larvae to determine the molt frequency and survival of successive stages after eyestalk ablation and removal of chelate appendages. Acceleration of molting was observed beginning with the second stage larvae. Eyestalk ablation has reduced the survival rate of the first and second stage larvae, but in the third, fourth larval stages and first juvenile stage survival is comparable to that of controls. Now that molt acceleration is demonstrated with eyestalk ablation, experiments are currently in progress to determine whether there is a corresponding weight gain in molt accelerated juvenile lobsters.

085

THERMAL COMPENSATION IN PROTEIN AND RNA SYNTHESIS DURING THE INTERMOLT CYCLE OF THE AMERICAN LOBSTER, HOMARUS AMERICANUS

John F. McCarthy*
Graduate School of Oceanography
University of Rhode Island
Kingston, Rhode Island 02881

The *in vitro* rate of incorporation of ^{3}H-leucine and ^{3}H-uridine into the acid-insoluble fraction and the concentration of RNA was measured in the hepatopancreas, abdominal muscle, and gill tissue of the American lobster, Homarus americanus, in the intermolt and premolt stages of the molt cycle and acclimated to warm (20°C) and cold (5°C) temperatures. Tissues of intermolt lobsters exhibited an adaptive translational acclimation to altered temperature, with respect to leucine and uridine incorporation. Hepatopancreas and muscle from premolt lobsters exhibited no acclimation or inverse acclimation. Gill tissue from premolt lobsters respond to temperature acclimation by rotation of the R-T curves for leucine and uridine incorporation. Most acclimation-induced alterations in the rates of leucine and uridine incorporation are completed within 10 days at the new temperature. Uridine incorporation in intermolt hepatopancreas required more than 10 days to readjust after transfer from cold to warm temperatures. Both acclimation and molt cycle condition alter the concentration of RNA in tissue. Premolt hepatopancreas had 40-60% more RNA than intermolt tissue. The intermolt cycle had less affect on the RNA content of muscle and gill. Cold acclimation of intermolt and premolt lobsters resulted in an increase in the RNA content of muscle and gill and a decrease in hepatopancreas RNA.

(Abstract of a paper submitted to Comp. Biochem. & Physil. for publication, 1975)

* Present address: Boston College, Boston, Massachusetts

46

THE INFLUENCE OF CNS FACTORS ON TEMPERATURE ACCLIMATION DURING THE MOLT CYCLE OF THE AMERICAN LOBSTER, HOMARUS AMERICANUS

John F. McCarthy* and A. N. Sastry
Graduate School of Oceanography
University of Rhode Island
Kingston, Rhode Island 02881

Injection of saline extracts of eyestalks and supraesophegeal ganglia from cold-acclimated lobsters into warm acclimated animals caused enhancement of the *in vitro* rate of incorporation of ^{3}H-leucine and ^{3}H-uridine within 12 hours of injection. The reciprocal set of injections depressed the incorporation rate. Mediation of the acclimation response by CNS factors is suggested. Eyestalk extripation in premolt lobsters results in no change in the non-adaptive response to temperature acclimation. Intermolt lobsters accelerated into premolt condition by eyestalk removal exhibit an adaptive response to temperature acclimation. It is suggested that the non-adaptive response of premolt tissue is due to an inhibition in the production of the CNS acclimation factors as the animals enters into a premolt condition.

(Abstract of a paper submitted to Comp. Biochem. & Physiol. for publication, 1975.)

* Present address: Boston College, Boston, Massachusetts

087

PYRIMIDINE BIOSYNTHESIS DE NOVO IN THE AMERICAN LOBSTER, HOMARUS AMERICANUS

John F. McCarthy* and George Tremblay
Graduate School of Oceanography
and
Department of Biochemistry
University of Rhode Island
Kingston, Rhode Island 02881

The existence of complete de novo pathway for pyrimidine biosynthesis was demonstrated in vitro in the hepatopancreas and abdominal muscle of the American lobster, Homarus americanus. The rate of incorporation of $NaH^{14}CO_3$ into orotic acid was 25-30 o/o greater in tissue of warm-acclimated lobsters in the premolt stage of the intermolt cycle, compared to those in the intermolt (C_4) stage. Temperature acclimation had little effect on this part of the pathway in intermolt lobsters. The rate of incorporation of ($^{14}C_6$) orotic acid into the acid-soluble fraction of the hepatopancreas of warm-adapted premolt lobsters is twice that of intermolt lobsters. In muscle tissue, the intermolt cycle condition had little effect on the rate of incorporation.

(Submitted to Comparative Physiol. & Biochem. for publication, 1975.)

* Present address: Boston College, Boston, Massachusetts

688

LOBSTER [illegible]AVIOR IN A SEMI-NATURAL ENVIRONMENT

Stewart Jacobson, Joelle Atema and Lauren Stein
Boston University Marine Program
Marine Biological Laboratory, Woods Hole, MA

In January of 1974, we established semi-natural habitats in two 10 ft. diameter, octagonal aquaria, with five lobsters (Homarus americanus) each, and several Cancer irroratus, Anguilla rostrata, Pseudopleuronectes americanus, and Tautoglabrus adspersus. The lobsters, with respect to size and sex, were identical as possible between tanks, as were the numbers of the other species. The aquaria, which received ambient seawater, were arranged identically with an oyster shell substrate, and cement blocks, rocks, and ceramic pipes to provide a surplus of shelters. Observations from February through August were made both during the day, following feeding, and (using red light) just after sunset, when lobsters are active under natural conditions. Individuals were identified by day glow orange letters glued to the carapace. Types of behavior we were able to quantify included occupation of specific shelters, feeding, activity, and social behavior. Our goal was to combine advantages of a field study, ample space and a variety of species, with those of a laboratory study, permitting a greater degree of control over the lobsters.

In our large aquaria, the lobsters appeared to be much less agressive than generally has been reported. Aggression was most frequent during feeding. Observations at night revealed few encounters, and these were usually either one sided avoidance without pursuit, or mutual ritualized displays.

The lobsters in descending order of size were female, male, female, male, all adults, and a juvenile female. Neither an animal's size nor sex seemed to determine its relative dominance. For instance, females slightly smaller than certain males were often dominant to the latter. Dominance shifted somewhat between different animals during the study, and complicating this picture was possible territorial behavior in the larger individuals. In one tank, only the two adult females were territorial from February through mid-May, following which no lobster showed stability of residence. In the second tank, only one animal, a female, was territorial for more than several weeks, until early June, when the largest male established a reproductive territory lasting until the end of August. Even our large aquaria space may have been too limited for all animals to be territorial.

Lobsters appeared to lose their position in the hierarchy just prior to, and for up to a month or more following the molt. Such animals were often observed on top of shelters, in exposed locations, where other lobsters apparently did not harass them. Although captive lobsters are considered quite cannibalistic, we lost only one animal, a juvenile female, out of six molts.

Previous observations in our laboratory had established in Homarus americanus the existence of a female sex phermone, which we assumed acted as a long-range sex attractant. Instead, in our large aquaria, female lobsters about to molt sought out, took up residence, and actively courted the tank's largest male. The males were very non-aggressive toward these females, and yet during this period, made violent attacks against other males as well as

fish. In each case, following mating, the males retired to the shelter and fed on the soft shell. Cohabitation, in or around the males' residences, continued for several days following mating.

Interspecific relations between lobsters and the other species were mainly pacific, although predation on Cancer by H. americanus may have occurred. In summary, our observations suggest that mass culture of lobsters, together with other economically valuable species, is feasible of suitable space and shelter are provided.

089

Growth Observations on *Homarus americanus* in a Polyspecies Aquaculture System with *Crassostrea virginica**

Joseph S. Levine
Boston University Marine Program
Marine Biological Laboratory
Woods Hole, Mass. 02543

ABSTRACT

One of the major challenges currently facing lobster aquaculturists is the design of a culture system in which lobsters may be housed individually and yet fed with a minimum of effort. Most systems under study involve either individual maintenance of the animals immediately after 4th molt in containers with automatic feeders, or acceptance of a high rate of cannibalism and claw loss due to aggressive interactions in mass-rearing systems.

The current work was designed to evaluate a grow-out system in which individually housed lobsters would require no individual attention.

The Woods Hole Oceanographic Institution's Environmental Systems Laboratory was designed to grow oyster (*Crassostrea virginica*) on dense cultures of unicellular algae nurtured in seawater enriched with secondarily treated sewage effluent. As a result of high ambient food levels, the shellfish -- which are maintained in 3' x 3' screened trays stacked in 5' deep cement raceways -- produce large amounts of biodeposits consisting of both feces and pseudofeces. Sizeable communities of three genera of amphipods (*Corophium* sp., *Gammarus* sp., and *Jassa falcata*), the polychaete worm *Capitella capitata*, and (seasonally) spat of the mussel *Mytilus edulis*, appear spontaneously in the system and thrive on the accumulated biodeposits in the trays.

Preliminary studies indicated that *Homarus* could be cultured for at least

*This work was done in partial fulfillment of the requirements for the degree of Master of Arts at Boston University.

two months in groups of 10-20 in sealed oyster trays with no additional food supplement. The current work showed that after 10th stage, suddenly increased cannibalism made mass rearing unfeasible.

Animals maintained individually after two months' mass culture however, increased in weight 5-10 times (ex: 3.4-35 gms) in 6 months at average temperatures of 18°C. Animals maintained in individual compartments 2-4 to a tray doubled or tripled in weight in the same period. All of these animals remained vigorous and healthy throughout the experimental period. There was no mortality, and the animals retained 'wild type' coloration.

Larger (40-50 ml carapace length) animals did not fare as well under these conditions. Most survived, but growth was unsatisfactory.

Further investigations indicated that growth of the medium-large sized lobsters in this experiment was limited by food levels substantially below those which could be provided by placing the trays in sections of the raceway receiving direct and vigorous aeration. Additional water turbulence thus provided increased the numbers of the largest food species by at least an order of magnitude. It is believed that future work will prove these higher food levels more than ample to sustain good growth in larger animals, and in smaller animals held individually several to a tray.

Combining the requirements of Crassostrea and Homarus in culture, the following schedule for Lobster/Oyster polyculture is proposed:

1) Mass culture of 10-20 post larval Homarus with newly sorted found oysters in sealed trays. Innoculation with Capitella will speed establishment of these food organisms.

2) After two months, "thin and transplant" lobsters and resort and grade oysters. Lobsters should be isolated in individual compartments 2 or 4 to a tray. (Additional work is currently in progress to determine optimal density

of lobsters at this point to provide best growth/unit area. Several simple compartment designs are also under evaluation.)

3) After 6-8 months, thin lobsters further to 1 or 2 per tray and place with large oysters. Innoculate with Capitella and grow to marketable size.