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PACIFIC AQUACULTURE ASSOCIATION

FINAL REPORTS

1993-1994

Guam
Palau
Marshall Islands
American Samoa
Federated States of Micronesia
Commonwealth of the Northern Marianas Islands

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Acknowledgement

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Commercial Sponge Market Promotion

by
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Pohnpei Natural Products

and

Dr. John Brown
College of Agriculture and Life Sciences
University of Guam

Final Report for the Pacific Aquaculture Association

April 15, 1994

Final Report to Pacific Aquaculture Association

April 15, 1994

I. PROJECT TITLE: Commercial Sponge Market Promotion

II. PROJECT INVESTIGATORS:

Mr. Richard Croft, Pohnpei

Dr. John Brown, University of Guam

III. PROJECT PURPOSE:

To promote commercial sponge marketing to tourists within the region.

IV. INTRODUCTION

Sponge farming is considered to be an attractive alternative for copra production on the isolated atolls of Micronesia. And like copra, once harvested sponges store well, so the physical isolation of the atolls presents little problem. Sponge farming is also suitable for the lagoons of the high islands. As an agricultural product, its production requirements fit well within the island cultures which may require the farmer to give his or her attention to social and cultural affairs for several days at a time.

Prior to 1940, the Japanese in Micronesia experimented with culturing commercial sponges (Cahn 1948). One document indicates the sponges used were found already growing in the various lagoons (Cahn 1948). A Mr. Smith sent a sample of the Micronesian sponge to Dr. M. W. de Laubenfels, a noted sponge biologist, and Dr. de Laubenfels reported "The specimen is spongia officinalis, subspecies mollissima, known as Fine Levant, or Turkey Solid. One expects to find this exclusively in the eastern Mediterranean, and it is absent or rare elsewhere in the world. The specimen is one of the finest I have ever seen. Its tubers are a little bit weak, perhaps as a result of chemical bleaching, but in general it is worthy of enthusiasm. This is the sort of sponge that is suitable for some of the highest-price use, such as for blood absorption in surgical operations. I have tested it carefully; its elasticity, holding power, and all-round "spongeness" are superlative. During mid-1987, Mr. Mike Gawel, FSM Division of Marine Resources, sent a sample of the sponge found in the Pohnpei lagoon to Gerald Bakus, University of Southern California. Professor Bakus responded "The sponge you sent me from Pohnpei, seems to be the worldwide commercial tropical sponge, Spongia officinalis, L. The taxonomy of Spongia is in poor shape and a revision of the genus is needed" (Gerald Bakus, personal communication).

V. Commercial Sponge Markets

Worldwide the demand for natural sponges continues to be high. Natural sponges are primarily used in industry (as lubricant applicators), by printers (to clean presses and

plates), by artisans and craftsmen, for applying and removing cosmetics, for bathing, and for general household use. However, the world production for all species of commercial sponges has dropped from an average of 1,346 tons per year from 1927 to 1936 to an average of 222 tons per year from 1977 to 1980, per the UN/FAO report "Sponges: World Production and Markets (Josupeit, 1988). In recent years, major sponge supplies have been from the Mediterranean, but blight is seriously damaging these sources. Natural sponges command a high retail value (from $4 to $60 each depending size and species) in part because of this large, unmet, demand. How ever wholesale prices are considerable less than retail prices.

Prior to the beginning of this project, wholesale price information was obtained by using cleaned, two year old samples of the Pohnpei sponge provided by Mr. Croft's private farm. One potential buyer, Clipper Mill in San Francisco, quoted a price of $1.00 to $1.25 per sponge for the five inch sponges. They went on to say "Once you have production of 500-1000 sponges per month, I would be willing to return to help get your commercial sponge industry started (Clipper Mill, personal communication).

More detailed information was obtained through Florida Sea Grant agent John Stevely. The sponge buyers in Tarpon Springs that he showed the samples to quoted a similar price as Clipper Mill, i.e. $1.00 to $1.25 per sponge for a steady supply. Stevely states, "In general the Tarpon Springs sponge buyers were impressed with the potential quality of Pohnpei cultured sponges. The most influential international buyer and seller indicated that he would be interested in handling a quantity of at least 10,00 pounds (Approximately 200,000 pieces). Based on the comments I heard, I believe it is reasonable to assume that the world sponge market could easily accommodate sufficient Pohnpei cultured sponge production to support numerous sponge farms. Stevely further states, "One of the Tarpon Springs sponge buyers has offered to buy 1,000 sponges for $1,000 so that he can conduct a market survey throughout Europe. This buyer has also expressed an interest in traveling to Pohnpei in the event that I was making a return trip" (Stevely, personal communication).

However, commercial sponge farming is in its early stages of development in Micronesia. The first commercial farm, Mr. Croft's, started harvesting and processing sponges in 1992 with production less than 1,000 pieces in the first year. Others are expected to initialize harvesting in 1994, and production will increase slowly. During this initial period of commercial sponge production in the Federated States of Micronesia, the quantities produced are considered to be too small to warrant sending to the large wholesale markets in the US. Their minimum quantity requirements ranged from 12,000 to 100,000 pieces per year. Prices quoted ranged from $US 1.00 to 1.50. In the next few years the numbers of cultured sponges produced in Micronesia will be most suitable for a smaller but higher priced market which is believed to exist among the hundreds of thousands of tourists visiting the region each year.

VI. PROCEDURES AND PROGRESS OF THE PROJECT:

Marketing to the regional tourist trade was seen as offering a viable alternative to the bulk wholesale market. Regional tourist centers on Guam and Saipan were targeted for development. The first step in this process was teaching the marketing person (Brown) about sponge farming, processing, packaging and uses of sponges. This was accomplished by Brown traveling to Pohnpei in September 1992. On Pohnpei, visits were made to the sponge farm and training was provided on the various types of sponges, on the normal processing procedure used in Pohnpei and a review was made of the existing marketing technique.

The second step of the project was to have been the designing of a test market and promotional campaign directed at appropriate tourist souvenir shops in Guam and the Northern Marianas. However, the primary marketing effort focused on the development processing and of packaging materials and labels for the sponges. This was done because the current packaging was not suitable to the Japanese gift market. The sponges were sold in an unbleached state to the local market in limited quantities. They were packaged in a plastic mesh bag with a small label stapled to the top. The label had a single hole punched in it to allow the package to be hung from a peg-board type retail display.

The process used to clean the sponges was found to have no problems, but it was decided to add a chemical bleaching step to the process. The chemicals necessary for bleaching were obtained form off-island sources, and a chemical bleaching technique was worked out for the sponges on Guam. The bleaching left an odor of permanganate in the sponges, so additional set of rinses which included the use of a fabric softener in the final rinse was added. This cured the odor problem.

A local artist was recruited to draw the art work for the package labels. The first artist completed a set of preliminary sketches for the labels. She then disappeared from the project. A total of four months was lost to the project while contact was made with the artist and a determination was made that she was no longer interested in participating. A second artist was contacted and preliminary sketches were again drawn for the labels. The drawings were refined, and they were printed in four colors. These initial labels are shown in Figure 1.

Concurrently, the actual packaging was being developed. While visiting Taiwan for other purposes, one of the investigators obtained a number of clear PVC tubes in three sizes from a local manufacturer. He also obtained cleaned and epoxied abalone shells for a second packaging design. A local woodworker on Guam was contracted to make wooden bases for the abalone shells. The student helper on the project then epoxies the shells to the wooden bases. Latter during a visit to Hawaii, he obtained three sizes of PVC corsage boxes from a floral supply shop. Contact was made with the local soap manufacturer in Pohnpei to obtain a supply of woven pandamus boxes.

Thus, four package designs were developed: 1.) a clear PVC tube in three sizes, 2.) an abalone shell mounted on a wooden base, 3.) a clear corsage box in three sizes, and 4.) a woven pandamus box. The pandamus box design was not able to be carried beyond this stage as we not able to obtain sufficient quantities for use in the test marketing. Samples of the three remaining package designs have been provided to PA'A.

The next step in the marketing development was the production of informational and promotional materials on the Micronesian cultured sponges. This was done by designing a package insert explaining the uniqueness of the farm-raised product. A first draft was produced in Pohnpei. This design was then modified and translated into Japanese on Guam. An initial press run was ordered for use in the test marketing. A sample is included as Figure 2.

Sufficient raw sponges were ordered from Pohnpei to provide materials for the test marketing. The initial shipment was hand carried to Guam by Croft at the time of the initial testing of the chemical bleaching process. A this time, a size grading scheme and pricing schedule were developed. Over the course of the project a total of 433 sponges were obtained. The size categories and their prices are shown below:

Table 1. Raw sponge prices to the project

Size	Category	Unit Price	Number	Value
< 4 inches	small	$2.00	123	$246.00
4 to 5	medium	2.50	152	380.00
5 to 6	large	3.00	106	318.00
6 to 7	x-large	4.00	28	112.00
>7	jumbo	6.00	24	144.00
Totals			433	$1200.00.

Thus, the average price of the sponges given the size distribution and pricing was $2.77. This is significantly greater than the $1.00 to $1.25 quoted by the mainland sponge buyers.

Once the sponges were obtained, cleaned and bleached, the inserts and labels printed and the whole assembled and packaged, test marketing was ready to begin. Prior to this project one of the investigators (Croft) had visited a local fair on Guam, and trial sales of Micronesian cultured sponges brought a good response at $6 to $7 each from local residents. However, tourists were not involved in these trials. The other investigator returned to the same fair with the product in May of 1993. Retail prices were as follows:

Table 2. Retail prices at the Micronesian Island Fair

Size	In tubes or boxes	In shells
Small	$10.00	$20.00
Medium	12.50	22.00
Large	15.00	
X-Large	20.00	

The Pohnpei sponges sold well, although many of the consumers were not familiar with a "natural" sponge or with its uses. Sample sponges were displayed wet to allow the customers to familiarize themselves with the product. Potential customers would normally ask several questions about the sponges before they would pick it up to feel it. Once they had felt the sponge, their next action would be to smell it. Then, they seemed to consider wither or not they were interested in buying one or more. Again many of the customers were not tourists, although there were a small percentage of tourists among the customers.

The overall impression gained from this initial test marketing was, first, that the sponges were very marketable if properly presented. Second, the majority of purchasers said that they were buying the sponges as gifts. Third, the general populace is no longer familiar with a natural sponge and its uses. They needed some explanation before they would buy it. Finally, the customers seemed fairly evenly divided between those that were buying it as a locally produced novelty and those that were buying it as a luxury gift.

The final stage of the project was the investigating and evaluating potential sponge markets and buyer and retailer responses to various forms and prices of sponges. As a prelude to this stage, the investigators (during travels for other reasons) investigated retail stores in Japan, Taiwan and the mainland US. A limited market search in Japan found 5 inch sponges retailing for 3,000 to 3,500 yen or about $22.00 to $26.00 in plain unmarked packages. Much smaller, 2-1/4 inch sponges were being sold for 500 yen or $3.70 for the removal of make-up. In Taiwan, bath sponges retail for US$ 10.00 for a 4 inch sponge to US$ 40.00 for the larger sizes. In the mainland US and on Guam, the retail prices of bath sponges ranged from $ 14.50 to $ 42.50. This combined with the initial test marketing on Guam gave strong indication that the initial assumptions of this project that the highest priced outlet available to the Pohnpei Sponge Industry is the gift market in Guam and Saipan was correct - provided that proper marketing development occurs.

After the test marketing, an initial visit was made to a local retailer to start the market introduction process. On this first sales visit, the retailer turned out to also have a wholesale distribution company. At this time, he expressed his interest in handling the entire distribution of Pohnpei sponges on Guam. Eventually, a verbal contract was

agreed to between the Pohnpeian sponge farmer and the wholesaler to purchase all of the farmer's current production. At this time, the University of Guam's role in the project was essentially completed.

The first round development of the packaging materials and art work was then completed, and the final versions were printed. These versions are shown in Figures 3 and 4. These were turned over to the farmer for his use in the Pohnpei tourist market. A second version of the flyer was provided to the wholesaler on Guam for use in the Mariana Islands

VII. EVALUATION OF ACCOMPLISHMENT OF MILESTONES AND GOALS:

Milestones:

A. Guam personnel will be taught about sponges and their culture in Micronesia. Accomplished.

B. Marketing promotion and monitoring strategies will be developed. Accomplished.

C. Suppliers of packaging materials will be contacted for assistance in developing suitable packing methods for our product. Promotional materials will be produced. Accomplished.

D. Sponges will be processed using several different methods and samples from each batch will be sent to agents on Guam. Accomplished.

E. The UOG agent will obtain responses from tourists and from tourist related businesses in Guam and the Northern Marianas.

Partially accomplished. Some tourists were interviewed at the Micronesian Island Fair, but this step was curtailed when the verbal contract with the local wholesaler on Guam was established. No further intervention by the University of Guam was desired or warranted after this.

F. Reports will be submitted quarterly and a final report will be submitted at the end of this project. Accomplished.

Benefits:

This project helped to promote the establishment of a viable commercial sponge aquaculture industry within the region. Development of the local market increased the price received by the farmer to an average of US $2.77 per sponge, given the size distribution of the first year's harvest.

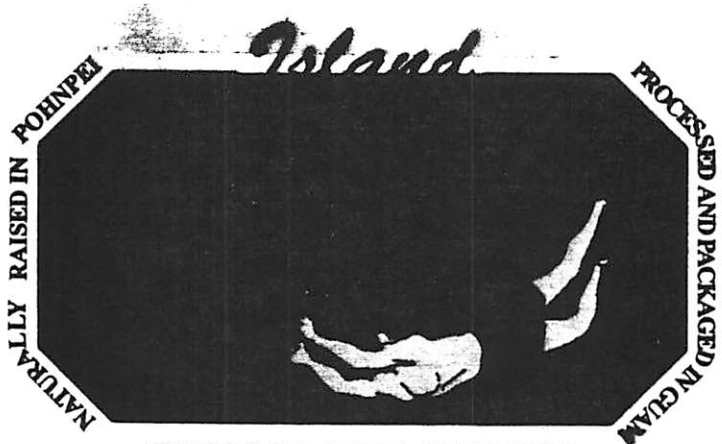
REFERENCES

Cahn, A. R. 1948. Japanese sponge culture experiments in the South Pacific Islands. Fisheries Leaflet no. 309, U.S. fish and Wildlife Service, U.S. Department of the Interior, Washington D.C.

Josupeit, M. 1988. Sponges: World production and markets. Draft report to the FAO of the United Nations. Rome.

Stevely, J.M.1989. Unpublished manuscript. Interim report on the potential for commercial sponge farming in Pohnpei, FSM. Prepared for the Marine Resources Division, FSM. Florida Sea Grant Program, sponsored by the Pacific Island Network (PIN).

FARM RAISED SPONGE



FARM RAISED SPONGE

NATURAL "WOOL" —海綿—

昔から海綿はその用途の多さから広く人々に親しまれてきました。しかし、近年ではその成長の遅さゆえ天然のものを集めるのはきわめて困難になってきました。そこで、1986年私達はポナペ島を選び、世界で唯一人工的に海綿を育てることに成功しました。これらの海綿はその特徴として、科学的に合成されたものより優れた耐久性、吸水性、又は無数に開いた穴により、洗剤等が海綿内に残りにくいことがあげられます。

尚、塩素漂白剤、ガソリン、又は酸性の強い薬品等は、海綿を傷める恐れがありますのでご注意下さい。

この海綿の販売は、下記の二団体が共同で行ないます。

Pohnpei Natural Products
P.O. Box 428
Kolonia, Pohnpei, FM 96941

University of Guam
College of Agriculture
and Life Sciences
Mangilao, Guam 96923

Natural "Wool" Sea Sponge

Natural sea sponges have been used throughout the ages. Until now, all natural sponges were collected from the wild. As sponges are very slow growing, the wild populations have been over-harvested throughout the world. However, the sponge in this package is from a sponge "farm". It was grown from a small cutting and took two to three years to reach this size. Pohnpei Island is the only place in the Pacific Ocean, and maybe in the world, where sponges are being cultured. The first small "sponge farm" was established in late 1986 and has been slowly expanding. Natural sponges are more absorbent and durable than synthetic sponges, and because of their interconnected holes, they will rinse cleaner. Harsh chemicals such as chlorine bleach, gasoline and strong acids will damage both synthetic and natural sponges.

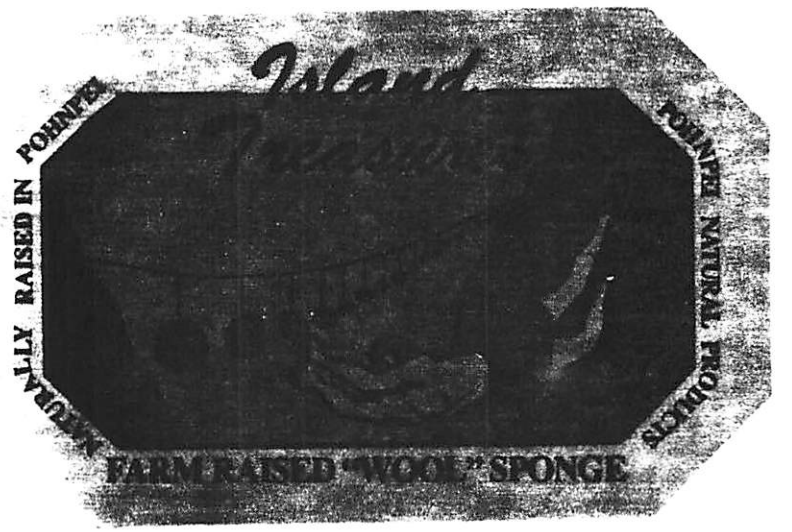
The marketing of this sponge is a co-operative project between:

Pohnpei Natural Products
P.O. Box 428
Kolonia, Pohnpei, FM 96941

and

University of Guam
College of Agriculture
and Life Sciences
Mangilao, Guam 96923

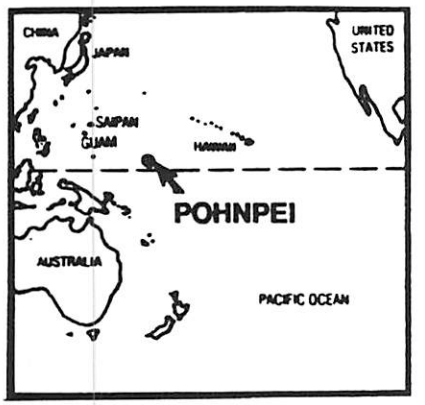
Island Treasures
Naturally Farm-Raised
SPONGE
Prpduct
of
Pohnpei
Pohnpei
Natural Products
P.O. Box 428
Kolonia, Pohnpei,
FM 96941


Island
Treasures
NATURALLY RAISED IN POHNPEI
POHNPEI NATURAL PRODUCTS
FARM RAISED "WOOL" SPONGE

Naturally Raised In Pohnpei
Island
Treasures
FARM RAISED "WOOL" SPONGE

Produced and Processed By:

Pohnpei Natural Products
P.O. Box 428
Kolonia, Pohnpei, FM 96941



Natural "Wool" Sea Sponge

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MARKETING GIANT CLAMS IN THE LOCAL FOODSERVICE INDUSTRY: A CASE STUDY

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This project was endorsed by the Pacific Aquaculture Association (PAA) whose mission is to promote and assist the development of aquaculture as a vehicle for economic and social advancement in the Pacific Islands with PAA membership.

The authors wish to thank the three restaurant owners in Pohnpei for their cooperation and generous donation of time. Special thanks to Dick Croft for his advice and help in making this project successful.

EXECUTIVE SUMMARY

This report describes the results of a case study in which a marketing plan for introducing giant clams into foodservice was developed, evaluated through a market test, and revised. The plan specifies actions corresponding to six key stages of marketing including market analysis, handling and distribution, pricing, promotion, service, and evaluation. The focus of the report is on the process of designing and implementing this plan. Although the activities and results are specific to the FSM Aquaculture Center in Kosrae, they are likely to be relevant to most giant clam aquaculture operations in the Pacific.

Results

Restaurant owners in the Pohnpei market were unfamiliar with giant clams, but enthusiastic about trying them. They were primarily concerned about handling and distribution. To address their concerns and ensure product quality and consistency, a protocol was developed and tested for selecting and shipping the clams.

The biggest challenge in developing a market in Pohnpei was in setting up an effective distribution system. It required finding someone who was willing to pick up clams from the airport terminal, break up the shipment and deliver the clams to the restaurants on a weekly basis.

The merits of various pricing policies were analyzed and assessed indicating that at this time, pricing should be based on a norm set by a regional price leader. Promotion actions included interviewing restaurant owners to find out their general reactions to clams, concerns and service needs, offering samples of clams to the restaurant owners, and providing them with table tents to encourage customers to order the clams. Because of the perishability of the clams, services were especially important, and restaurant owners were assisted in making appropriate holding arrangements for the clams and were given demonstrations on clam cleaning and sashimi preparation.

Recommendations

Based on the results of the market test, the following revisions and additional actions are recommended for enhancing the effectiveness of the marketing plan:

-- Clams should be consistently sized in the five to six inch range.

-- The distribution system should be carefully monitored to ensure adequate functioning.

-- The feasibility of forming a marketing cooperative to stabilize price and strengthen marketing capabilities should be investigated.

-- Research should be directed toward developing a quality frozen clam product and on maintaining the quality and extending the shelf life of fresh shucked clams.

-- Table tents should continue to be the major form of advertisement, specifying that the clams are "baby giant clams," that they are cultured, and that customers may keep the shells as souvenirs.

-- Consumer acceptance of the clams and satisfaction among restaurant owners and managers should be assessed regularly.

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MARKETING GIANT CLAMS IN THE LOCAL FOODSERVICE INDUSTRY: A CASE STUDY

INTRODUCTION

During the past decade the number of giant clam operations in the Pacific has doubled; presently there are over twelve giant clam hatcheries in the Pacific islands and more are planned. One of the most important goals of these projects is to create new economic opportunities for the local population. But to encourage the islanders to invest their resources in clam aquaculture, the economic viability must be demonstrated. This can only be accomplished by establishing profitable markets.

Research in giant clam production has greatly assisted the development of the industry but more resources must now be directed to marketing. The production of clams continues to increase creating a great imbalance between supply and demand.. As a result of this imbalance, the Pacific giant clam industry is at a critical stage of its development. To help ensure that the vitality of this fledgling industry is not seriously threatened, reliable markets with long term growth potential must be established now.

Marketing studies to date have concluded that the largest potential demand for giant clams is for food; the foodservice market in particular is an especially viable target (Shang, et.al., 1990; Tisdell and Kuronuma, 1992, 1993). Preliminary studies further indicate good prospects for fresh giant clams in major foodservice markets such as Hawaii and Guam (Shang, et. al., 1992). The large tourist base in these areas is likely to correspond to a healthy demand for more exotic or luxury food items (Tisdell, 1989). But in addition to overcoming the institutional impediments of penetrating and developing these markets, successful entry will require sophisticated market planning. This sophistication can best be acquired by concentrating initially on local markets and helping local clam producers gain experience, knowledge and expertise in market planning and development.

Background

The Giant Clam Hatchery in Kosrae, FSM, began raising clams in 1991. Like other Pacific hatcheries, operations have grown and further expansion is planned. Tridacna derasa is the predominant species reared at the Center. Sensory tests indicate that this species is highly suitable for food consumption (Peavey and Riley, March 1993; Peavey and Riley, July 1993). However up until late 1992, marketing efforts were virtually nonexistent. With market prospects lagging far behind production capacity, the Center's development priorities shifted toward marketing. The local FSM region seemed to hold the best opportunity for initiating work in this direction. To successfully introduce clams and maintain this market, a formal marketing plan was needed to specify the best procedures for accomplishing all essential marketing actions.

Contrary to common perceptions, these marketing actions encompass far more than just advertising and selling; they cover every action that occurs in moving the product from the point of production to the point of consumption. Even in targeting the most accessible, local markets, the proximity of producers to the final markets, the extreme perishability of the product, the diversity of the end-user needs in terms of handling assistance and demand, all create a complicated set of variables that need to be accounted for.

Moreover, cultured giant clams as a food product for foodservice are essentially new to the market (Tisdell, 1992; Tisdell and Kuronuma, 1992). As such, new products often encounter buyer resistance because they require considerable investment and risk on the part of the buyer (Gibson, 1986). Especially with a product as perishable as giant clams, market introduction requires the development of adequate arrangements and timing in handling, distribution, storing and preparation. These factors had to be clearly addressed in the marketing plan to reduce the perceived risk to prospective customers.

This document is a case study which describes the design and results of a plan for developing a local foodservice market for giant clams. Based on the experiences gained through this study, it is intended to be an applied, straightforward guide which

addresses the practical questions of marketing typically faced by giant clam producers. As a result, it is meant to be a useful addition to the marketing knowledge of those involved in Pacific giant clam aquaculture.

Objectives

The study was designed to meet six objectives:

1) To identify the potential customers for giant clams in the local market and evaluate their product and service needs.

2) To establish a protocol for handling, shipping and distributing giant clams.

3) To select a pricing policy.

4) To develop a promotion and service plan.

5) To evaluate consumer acceptance of giant clams.

6) To evaluate the marketing plan, recommend actions to enhance customer satisfaction and promote market stability.

PROCEDURES

This guide details the results of a case study in which a marketing plan was developed, evaluated through a market test, and revised. The plan was designed to achieve each of the six objectives. The actions detailed in this report were found to work best for the National Aquaculture Center in Kosrae for developing the local foodservice market. Although the activities and results are specific to this particular operation, they are likely to be generalizable to similar giant clam aquaculture operations.

Corresponding to the first objective, the best prospects for concentrating marketing efforts were identified by evaluating the size and characteristics of the foodservice industry within the four FSM states, selecting a sample of restaurants, visiting these businesses and conducting a menu census. Based on menu prices, clientele and type of menu offerings, restaurants were evaluated and owners of selected establishments were contacted for interviews to identify their concerns and information needs, their familiarity with clams, product and service preferences, and to assess their willingness to try a new product.

Product handling procedures for fresh giant clams were tested and evaluated to meet the second objective. To ensure that restaurants were supplied with the best possible product, information was needed on the shelf life of fresh giant clams and effects of shipping time and temperature to which the clams are subjected. Shelf life studies and sensory analysis were performed to analyze the effects of different handling procedures on product quality. This determined how frequently a restaurant should be supplied and how they should handle and store the clams.

Methods for addressing objective two included identification and assessment of available distribution channels in the target area. Air shipment is the only alternative for perishable products such as fresh giant clams. However, air freight minimum requirements and fees have a significant impact on price and the necessary distribution requirements. Options for delivery from the airport to the customer were identified and evaluated for feasibility, cost and reliability. The intent was to identify the best distribution system and place this channel into operation. This entailed negotiating who would act as a distribution agent, what that person would do and receive for performing specific marketing functions.

A pricing strategy was devised by reviewing previous market studies, considering production, shipping and distribution costs, the nature of the industry and obtaining information on current pricing standards. A promotion strategy was designed to help restaurants encourage their customers to order clams by reaching customers with information on the product. Interviews with restaurant owners clarified what type of information was likely to be most effective and also indicated what services would help assure their satisfaction and the satisfaction of their customers.

The final objectives were met by instigating and analyzing a market test conducted in selected foodservice operations in the target market using the strategies and actions specified in the marketing plan. During March 1993, selected restaurants were given fresh giant clams to menu. They were allowed to choose whatever preparation they wanted and to sell them at any price. In return, the restaurants asked customers who ordered the clams to complete a customer satisfaction survey. They

also kept records of sales, preparation and menu price. The test marketing was done to fine-tune the marketing plan; to identify weaknesses and suggest ways it could be strengthened, to identify problems, find solutions and generally reduce the likelihood of failure in establishing this market and future prospects.

At the conclusion of the market test, interviews were held with the restaurant owners to discuss their price expectations in both purchasing and menuing the clams. Based on the optimal shipping and handling procedures, distribution, potential demand, storage, menuing and preparation, all related costs of marketing were identified. This information was used by producers, marketing agents and restaurant buyers to refine their price and demand expectations. In addition, owners were asked about their level of satisfaction, any problems or concerns and their suggestions for revising the marketing plan. Their feedback indicated what actions should be taken to ensure customer satisfaction over the longer term, to maintain loyalty from these buyers which in turn would promote market stability.

It is important to understand that this report, written as a case study, describes the exact procedures that were followed. During the process of designing and implementing the marketing plan, mistakes were made, certain aspects could have been handled more efficiently, certain assumptions were incorrect, some actions prescribed in the plan were inadequate in meeting the objectives. This illustrates the essential role of the market test which allowed an objective appraisal of the plan. On this basis, the plan was revised and these details are included in the recommendations section of the report. This report elaborates on the mistakes as well as the successes so that other market developers may be deterred from following similar ineffective procedures and can adapt the procedures to fit their own marketing instincts.

DEVELOPING THE MARKETING PLAN

The market plan consisted of a five stage process which addressed all objectives. This is shown in Table 1.

Table 1. MARKET PLANNING DETAIL

Planning stage	Program
I. Market Analysis	--Define the targeted foodservice market based on region, customer base and characteristics of prospective restaurants --Interview potential customers to identify product and service needs and the best prospects for marketing efforts
II. Handling & Distribution	--Identify and evaluate handling and shipping options Based on available resources and the product needs of potential customers --Identify and evaluate options for receiving and delivering product to customers based on available resources, product, and service needs of potential customers
III. Pricing	--Determine current price norms for the industry --Evaluate nature of the industry --Identify all costs as accurately as possible --Develop pricing policy
IV. Promotion and Service	--Develop ways of effectively presenting information on the product to restaurant owners and their customers --Develop policies for billing, handling customer problems and complaints
V. Evaluation	--Conduct market test and consumer survey to obtain feedback on all procedures, buyer satisfaction and consumer acceptance. --Analyze results, revise pricing and promotion policies, delivery schedules and service needs.

Market Analysis

The market analysis served as the framework within which the entire marketing plan was based. The first step was to evaluate the local region and identify a specific target for concentrating marketing efforts. The evaluation was based on information gleaned from earlier marketing studies. Most of these studies indicated that the Japanese market as well as the island tourism market represent good prospects for selling giant clams (Dawson, 1986; Shang, Tisdell and Leung, 1990; Tisdell and Kuronuma, 1992). These two considerations alone indicated that within the FSM region, Pohnpei was the best location. This FSM state has the highest number of

visitor arrivals, consisting of a large proportion of Japanese visitors, and it has the most developed tourism industry (Office of Planning and Statistics, 1991). Correspondingly, it also has the largest number of foodservice establishments.

Identifying prospective customers.

The next step was to identify those restaurant owners who were likely to be most interested in buying giant clams. The best prospects were those in which the characteristics of giant clams complemented the characteristics of the restaurants.

Several distinct features of giant clams were considered in identifying the best sales prospects: clams are a specialty seafood item and would require special handling and preparation. The unit costs of clams are fairly high relative to other standard feature items on local menus, such as chicken or tuna. The clams are also likely to have particular appeal to visitors, especially Japanese tourists. On this basis, the key was to locate restaurants that featured seafood, had menu prices in the mid to expensive range, had a solid tourist/visitor clientele base, and employed staff who were experienced in seafood.

Information on the characteristics of specific restaurants was obtained by talking with people who were familiar with Pohnpei. This group included business travelers, tourists, travel agents, and people who live in Pohnpei. Of the 10 restaurants visited, four were identified as good prospects. Three of the restaurants were owned by Japanese and featured Japanese cuisine. All catered to tourists, especially Japanese, and were reputed for the good quality of their food and operations. Overall menu pricing was within the moderate to expensive range.

Conducting the Informational Interview

The owners of these restaurants were contacted to schedule an interview to get information in three areas: 1) their familiarity and experience with giant clams; 2) their product preferences, concerns and willingness to try giant clams; 3) their suggested menu price ranges, expected margins and estimated demand.

The results of these interviews are summarized here from a more detailed report (Peavey and Riley, 1992). It should be noted that one interview was conducted

with assistance from an interpreter, since the Japanese owner and his chef did not speak English.

At the beginning of each interview, three live T. derasa clams ranging in size from three to five inches were presented and owners were asked for their reactions. All were genuinely interested in the giant clams and remarked on the attractive appearance of the product. Their experiences with clams varied from those who had never seen them, to those who had actually prepared and eaten wild giant clams. Nevertheless, familiarity was limited for the most part, and the clams were perceived as a new product.

All four indicated a preference for a four to five inch clam which they felt would be most appropriate as a sashimi appetizer. They were concerned that meat from a clam larger than five inches would be too chewy or tough. Without exception, the owners felt the clams were most appropriate for sashimi. They were only marginally interested in other preparations.

The owners said they would prefer to buy the clams fresh in the shell. Two owners felt that if their customers could keep the shell, this would enhance the appeal of the product, especially to tourists. They were also enthusiastic about using the shells for serving the sashimi; one shell half could hold chopped clam meat and the other half could hold the dipping sauce.

Because no one could think of any food item they were currently using or had used in the past that was similar to the giant clams, their ability to anticipate demand was limited. But all indicated that the clams would probably be popular among tourists because most of these customers were interested in local fare and exotic foods.

Their price expectations were remarkably similar. As a sashimi appetizer, they suggested a menu price of $4.00 to $5.00. In turn, most of the owners anticipated a wholesale price of $2.00 to $3.00. This was important information because the general guideline in foodservice is that the menued price of the featured or "center of the plate" item should be approximately 300 percent of its wholesale cost (Food Institute, 1990). In other words, if the wholesale price of a clam was $2.00, the

menued price as a sashimi appetizer should be $6.00 following the general rule of thumb. Based on the price expections they stated in the interviews, three of the four owners indicated they would be willing to work on a smaller margin.

Demand estimates varied, with individual predictions ranging from 20 to 350 clams per week during peak tourist months to a low of 5 to 10 clams per week during off tourist periods. When asked about their business cycle, they identified early May, August through September and early January as their heaviest tourist months. Consequently their food demand changed from month to month, but was somewhat predictable based on fluctuations in the tourist season.

Their major concerns centered on distribution, handling and storing. When asked about their usual methods of procuring product, they all reported that they picked up their own orders from the container ships, or they shopped for products themselves in various markets. Few, if any products were actually ordered and delivered to the restaurant premises. They were unanimous in their high appraisal of having someone deliver the products to them and they indicated they would be willing to pay for this service.

Depending upon the stability of the product, they preferred a weekly delivery. They confirmed they needed clear information on holding the clams and if they were to actually purchase clams, they wanted some assurance of product stability. They were also concerned about the reliability of the supply and wanted to know if they would be able to count on regular shipments. Also, because they couldn't anticipate demand, they felt it would be difficult for them to make accurate orders and they expressed reservations about a minimum order requirement that was much over 15 clams. On the other hand, they were also concerned about ordering too few clams and finding that they couldn't meet their own customers orders. In response to this problem, the owners were enthusiastic about the idea of using table tents to advertise and menu the clams to their clams, rather than actually adding the item to their menu. This would allow them flexibility in menuing the clams, based on their stock on hand.

The results of these interviews pointed to the following five fundamental actions that would help attract these prospective customers in this target market: 1) provide restaurant owners with concise storage instructions; 2) provide them with easy to follow instructions for shucking and preparing the clams for sashimi; 3) enable restaurant operators to offer clam shells to their customers; 4) ensure a reliable supply in terms of product specifications and distribution. 5) design table tents to inform customers of the product and allow the restaurant owners flexibility in offering the product.

Handling and Distribution

Restaurant owners were most interested in purchasing live giant clams in the shell. To ensure the quality of this product, the fundamental task was to design the best procedures for shipping and handling. High standards of control were especially important during this market introduction phase. The details of these standards had to be specified in a formal protocol. The packers would then know exactly what guidelines they were to follow in selecting and handling the clams for shipping.

There were three areas to address. First, the protocol for packing and shipping the clams had to be developed. Second, the shelf stability of the clams needed to be tested under conditions similar to what they would receive in storage at the restaurant. Based on these tests, the best handling procedures could be identified and a straightforward set of instructions could be developed that would be easy for the restaurant owners to follow and would ensure the quality of the clams. Finally, it was important to set a protocol for opening and cleaning the clams. Once again, these instructions had to be non-technical and easy to understand. The crucial consideration was that within this market, English was a second language to most of the restaurant owners and virtually all of their employees.

Air freight through Continental Air Micronesia was the only option for shipping clams from Kosrae to Pohnpei. Consequently, the methods selected for packing had to ensure survival while minimizing stress to the animals for at least five hours. This was the average amount of time the clams would spend in transit from the time the clams were first packed to when they arrived in Pohnpei.

Giant clams have an extremely short shelf life once removed from the seawater. Whereas shellfish from cold waters can be kept alive under refrigeration for some time, giant clams have an optimum water temperature range of 75 to 85 degrees F and refrigeration quickly kills them. Spoilage, at least as indicated by smell, begins rapidly. This may be due to decomposition of the zooxanthellae present on the mantle. Regardless of the cause, the result is a particularly offensive and pervasive odor. Shucked clam meat, even after removal of viscera and rinsing, begins to smell bad almost immediately if it is refrigerated.

With these considerations, packing procedures were tested and revised leading to a finalized protocol. Materials used include shipping boxes, plastic bags, approximately 2 to 4 mil, newspaper and oxygen. Shipping of giant clams is now routine at the Center. To date, more than 5,000 two year old clams have been shipped using this protocol with a total mortality of six clams.

Devising A Protocol for Packing and Shipping

On the day before shipment, the clams are selected for shipping. In developing the protocol, the two major criteria for choosing clams were the size and the appearance. A gauge was constructed so that packers could easily measure the clams. Based on the preferences of the restaurant owners during the trial stage, packers were instructed to select only those clams in the four to five inch range as indicated on the gauge.[1] This ensures product consistency, an important consideration expressed by restaurant owners who all said that one of their main concerns was portion control. After the clams are selected and detached from the raceways, the shells are scrubbed to remove algae and other creatures attached to the clam and to enhance the product appearance.

On the day of the shipment approximately two to three hours before flight departure, the clams are packed. First they are placed in layers of wet newspaper in a heavy plastic bag. This plastic bag acts as a liner for the shipping carton. The wet

[1] Based on the results of the consumer survey and their own experiences, restaurant owners later revised their size preferences to a five to six inch clam.

newspaper keeps the clams moist and also prevents the sharp shells from piercing the bag. Depending on the thickness of the plastic bags, double bagging may be necessary. The shipping cartons have a volume of approximately three cubic feet and were purchased from an aquarium/tropical fish supplier. A full carton holds up to 30 clams, each clam weighing about 1-1/2 pounds. Total box weight including packing materials ranges from 60 to 70 pounds. After the clams have been packed in the box, the plastic bag is filled with oxygen and then securely tied off with rubber bands.

Experiments were conducted at the Center to determine the maximum time the clams could be held this way before significant mortality begins. Simulated shipments of clams were left outside where the temperature ranged from 80 to 90 degrees F. One box was left out for 24 hours, then the clams were returned to the raceways. None of the clams died. In comparison, one box was left outside for 36 hours and resulted in 30% mortality. These results indicate that for any shipment taking more than 24 hours it is necessary to break the journey and put the clams back into suitable seawater for a few days. The logistics of doing this are obviously not easy.

In considering larger potential markets, clams could certainly be air freighted to Hawaii from Kosrae since the clams would usually spend not more than 14 hours in transit, based on the time at which they were packed at the Center to when they arrived at the destination. But a delayed flight could be disastrous. Since the Kosrae to Pohnpei flight is a direct one hour flight, the only concern was that there would be no errors in offloading the shipment from the flight.

Devising A Protocol for Storing the Clams

A second protocol was needed for holding the clams at the restaurant, since owners preferred to have deliveries once a week. These procedures had to ensure that the owners would be able to keep the clams alive until the time of preparation.

Tests were conducted on holding clams in small coolers which contained enough sea water to completely cover the clams. In one test, 10 two year old T. derasa were placed in a small cooler of approximately 2 cubic feet which was filled with seawater. The cooler was left outside of the Center in the sunlight with no water

exchange or aeration. Ten days later all the clams were still alive and healthy. After two weeks in this environment they began to show signs of stress and were replaced in the raceways; none of these clams died. Throughout this test, dissolved oxygen levels were carefully monitored. Levels ranged from 6.0 ppm early in the morning to 11.0 ppm at mid-afternoon.

It seems that photosynthesis by the symbiotic zooxanthellae on the mantle produces more than enough oxygen to support the needs of the clams, provided there is sufficient sunlight. Salinity ranged from 30 to 35 ppm on the bottom, to 20-36 ppm on the surface of the water. Fresh rainwater did collect at the surface, but then evaporated. Equipment for measuring ammonia levels was not available, but at no time did the water develop any off-odors. Temperatures rose as high as 92 degrees F and probably caused the stress which eventually terminated the experiment after two weeks.

This test indicated that clams can easily be held for several days in a container of sea water without water exchange or aeration, under three conditions: (1) densities are kept low enough so that all clams are fully exposed to the light; (2) the container is in a location with sufficient sunlight for photosynthesis without overheating the water and; (3) salinity is maintained at correct levels by replacing any evaporated loss with fresh water.

The experiment was repeated under different conditions. In one of these tests a small aquarium aerator was added to the container and the clams were kept inside by a window. After two weeks the clams remained healthy. These results indicated that the best solution to the live clam storage issue was to set up an aerated system in each restaurant.

Based on these experiments, researchers composed a list of instructions for the restaurant staff. The most important point was to inform the staff that in contrast to how they usually store seafood, the clams should not be refrigerated. The instructions explained the general biology and functioning of the clams and emphasized why it was important to adhere to the storage procedures. The instructions then specified that the clams should be placed in a single layer inside a large container, such as a large pan,

styrofoam cooler or ice chest, and covered with seawater. The container should be placed near a light source, but not in direct sunlight for the entire day since the water temperature could get too high. Restaurant owners were encouraged to use an aerator in the clam holding containers so that the water needed changing only once a week. If they did not use an aerator, they were instructed to change the seawater every two days. A copy of these instructions is included in Appendix A.

Another aspect of product handling pertained to the product preparation. During interviews, most restaurant owners indicated they did not know how to open the clams or prepare the meat. Moreover, they were interested in keeping the shells and wanted to know how to clean them. These procedures needed to be explained in a set of instructions that could be used by restaurant owners, chefs and any other staff who would be involved in food preparation. The general guide that was developed uses a series of photographs to illustrate and explain where to insert the knife, how to cut the adductor muscle for easily opening the shell, how to identify the edible clam meat, mantle and muscle, and separate these portions from the viscera which should be discarded. Directions for cleaning the shells in a weak solution of bleach are also included (see Appendix A).

Although sashimi was the preferred preparation choice among the restaurant owners, the marketing staff wanted to demonstrate the versatility of the clams and encourage restaurant owners to expand their usage of them. They compiled a list of preparation suggestions by searching through literature, seafood cookbooks, and getting ideas from people who were familiar with giant clams. These recipes were assessed for the ease in preparation, availability of ingredients, estimated costs, and sensory appeal. Eleven simple recipes were selected and distributed to restaurant owners with the instruction packets (see Appendix A).

Establishing a Distribution Channel

The term distribution channel refers to the series of actions needed to physically move the product from point of production to point of consumption. The distribution channel encompasses a number of functions typically performed by marketing agents who are often referred to as the "middlemen." Of particular interest

in this project was the pickup and delivery of giant clams at the point of arrival in Pohnpei.

The standard approach to choosing a distribution channel is to select the most timely and cost effective method of ensuring delivery while maintaining product quality. Because of their perishability, giant clams require the quickest and most efficient channel involving the fewest number of intermediaries (Shaw, 1990; Chaston, 1983). This helps limit the possibilities of delays associated with repeated changes in handling responsibilities.

The responsibility for rapid distribution to the Pohnpei market required the use of at least two intermediaries. Continental Air Micronesia was one intermediary, but a second agent was needed to distribute the clams once they arrived in Pohnpei. As straightforward as the problem sounded, this aspect of the marketing plan proved to be the most challenging issue because there was no distribution infrastructure whatsoever. In procuring most of their food products, restaurant owners acted as their own distributors in which they visited markets, purchased their selections, and brought them back to their business. These were time consuming activities, indicating the value of finding an intermediary who would act as distributor and assume these functions.

Another concern was that while air freight rates were based on weight at 12 cents per pound, Kosrae to Pohnpei, there was also a minimum charge of $20.00. This corresponded to a shipment of 167 pounds or about 70 to 80 clams; far more than a single restaurant owner was willing to purchase at one time. Individual buyers only wanted 10 to 15 clams. As a result, an intermediary was needed to break down the loads in Pohnpei into individual orders and deliver these to the restaurants.

Equally important to cost concerns, customer confidence in the product could be established not only on the basis of product quality, but also on the reliable performance of the distribution system. In the short run covering the market entry time period, the problem of insufficient volume would not only effect costs but would also impede development of this distribution system. The difficulty was in setting up

a reliable system that would only be used part time. This period could last up to several years before demand would become more predictable.

Based on these considerations, the person who assumed this responsibility had to be flexible and willing to work under sporadic market conditions. Yet at the same time, reliability was an essential concern; orders had to be broken down and delivered as soon as the shipment arrived. In addition, the distributor needed some fundamental knowledge of or interest in learning about giant clams to provide information and assistance to customers regarding the storing and handling of clams. In the ideal situation, the distributorship would best be assumed by someone who would see it as a venture that could create the opportunity for expansion and a more secure payback in the longer run.

A person was located who closely fit the profile of the ideal distributor for this particular market, and in addition, was experienced and knowledgeable about the care of giant clams. The next step was to negotiate the roles this person would assume in six key elements of establishing a distribution system: 1) physical handling; 2) ownership of the product; 3) payment for product; 4) promotion; 5) service and; 6) market information.

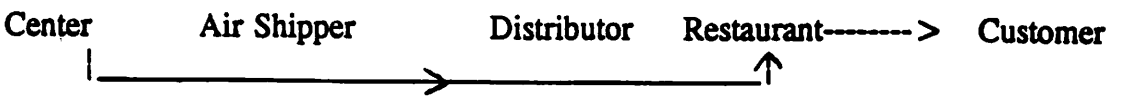
The design of the distribution system and the roles of each intermediary were finalized. This design evolved into what is defined as a five stage system consisting of the Center, the air shipper, the distributor, the restaurant, and the final consumer. Figure 1 shows a diagram of this system; it identifies all the agents involved in moving the product from producer to the final customer and clarifies their responsibilities with respect to the six key elements of the distribution system (Chaston, 1989).

Figure 1. Stages in the Distribution System and Roles of Each Agent

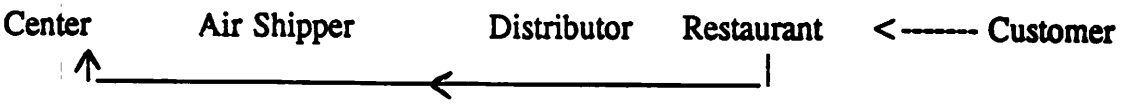
1. Physical Handling

Center-----> Air Shipper-------> Distributor--------> Restaurant--------> Customer

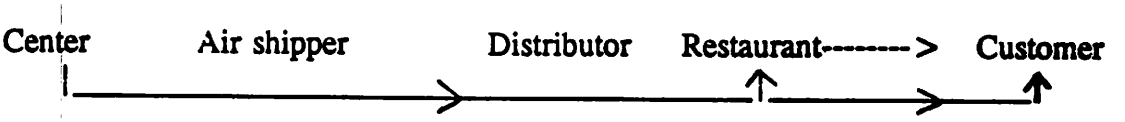
2. Ownership of the Product



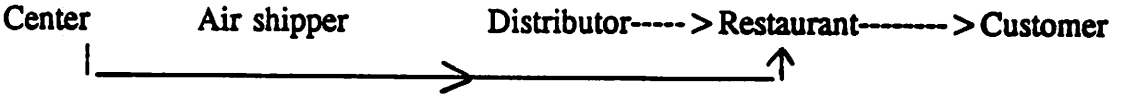
3. Payment for Product



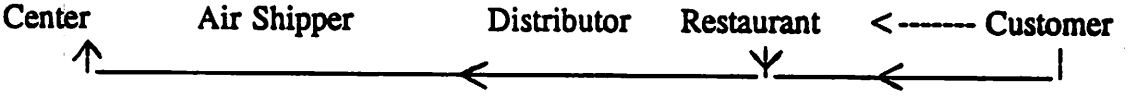
4. Promotion



5. Customer Service



6. Market Information



This diagram shows that for the most part the roles of the two intermediaries, the air shipper and the distributor, are minimized. Specifically, the responsibilities of the airshipper pertain only to the physical handling, encompassing nothing more than loading the clams at the terminal in Kosrae, transporting them to Pohnpei, and off-loading them at the terminal in Pohnpei. Upon arrival in Pohnpei, the distributor agreed to pick up the shipment, break it up into individual orders and deliver the orders to the restaurant. Outside of these actions, the distributor was cautious about committing too much time and responsibility to the project and consequently wanted

to minimize his involvement in the other five areas of the distribution system. With this in mind, Figure 1 illustrates that in terms of ownership, the product passes from the Center to the restaurant and then on to the final consumer. Similarly, payment for product follows a reverse pattern from customer to restaurant to the Center.

Promotion entails passing information about the product on to the customers. The restaurant assumes the major share of this responsibility since the menu itself is the most effective means of promoting the clams. The diagram also indicates that the Center would act as the major conduit for conveying information about the product that could be used in menuing and would instigate any additional advertisements or promotional activities directed to the final customers. The Center also would be solely responsible for developing a sales presentation and identifying and approaching other possible buyers.

The next consideration was customer service. The distributor agreed to provide a basic level of customer service by helping the restaurant owners find the best location for holding the clams. He also agreed to call all the restaurant owners once a week for their orders. Center staff would be responsible for contacting the distributor at a particular time to get the entire order from him. Other services, including assistance with clam cleaning and preparation, product guarantee and billing would be provided by the Center directly to the restaurant.

Market information consists of monitoring customer complaints, problems and overall satisfaction with the product. As shown in Figure 1, customers were most likely to direct their comments to the restaurant owners. In turn, the restaurant owners would pass information back to the Center about their customers' reactions as well as their own experiences with the product, again circumventing the distributor. The Center also planned on conducting a consumer survey as part of the market test; this would provide direct feedback between the final customer and the Center.

Based on these negotiated roles for the distributor in Pohnpei, reimbursement was the final issue. This payment had to be sufficient to cover the distributor's time, material and labor and ensure his continued participation in a market system that would only be used part time. The weekly time commitment based on the agreed

responsibilities was estimated to be no more than 3 hours. Transportation expenses were also estimated.

Several options were discussed and the distributor indicated a preference for compensation based on the number of clams delivered. Assuming that the farmgate price of the clam was approximately $2.00, a payment of $.35 per clam for pickup and delivery was agreed to as a reasonable fee; the fee for the distribution service was just over 15% of the farmgate price.

Price

Pricing decisions are usually made on the basis of a variety of factors, but the decision is usually founded on a predominant policy. Three of the standard policy options include basing price on costs of production, on knowledge of demand and competition, or on specific objectives of the business such as maximizing market share.

These major pricing policy options were reviewed for this project and the merits of each were assessed in terms of the status of available production and market information, and by considering the nature of the industry. On this basis, one directive guiding the selection of a pricing policy for the Pacific giant clam industry was obvious; producers should not attempt to buy their way into the market. To ensure the long term stability and vitality of the industry this is a crucial point to understand and to follow.

In evaluating the pricing policy options it became clear that for the giant clam industry in this region, information that is typically used by businesses to calculate a specific target price is extremely limited if not biased. For the most part the development of the giant clam industry in the Pacific has relied heavily on subsidies in the form of grants and other transfers rather than on private investment and entrepreneurship. These grants have been successful in helping new operations struggle through the precarious start-up stages of production. As a result, few, if any of these businesses have a thorough awareness of the true costs of production; for example, clam seed or money for buying seed, equipment and expertise have been donated to producers and their facilities. For many of these operations, cost-base

pricing is impossible to follow because the costs of production are either unknown or artificially low.

The second important consideration is that the product, giant clams, and more specifically, T. derasa, is likely to be relatively homogeneous; generally, it is difficult to distinguish between a five inch clam grown on one island versus a five inch clam of the same species grown on another island. Of course, service and delivery are other key factors that will help distinguish a product and supplier in the market place. But based on product alone no single clam seller in the Pacific is likely to be capable of establishing a price significantly higher than another seller without risking a total loss of customers.

This means that those operations supported by heavy subsidies have a distinct pricing advantage over those that are not; the subsidized producers could be tempted to establish a market by undercutting the price set by the non-subsidized competitor. This situation often leads to price wars, inducing operators to cut prices to a level well below break-even, the point at which they are no longer able to pay for their production costs. Over time, the operator who initiated the price cut will in turn face a new market entry from another competitor offering even lower prices. At this point the company will lose customers. Inevitably, the situation creates a severe financial burden and can lead to business closures or cutbacks (Chaston, 1989). At worst, it can lead to the dissolution of the healthiest and truly most competitive operations. Marketing among businesses involved in the Pacific Giant Clam industry is a tenuous if not dangerous proposition if producers do not understand or choose to ignore these points in establishing a price policy.

Optimally, the pricing policy is based on detailed information on costs and demand. But since information about production costs is vague, and information about demand is extremely limited in this introductory phase, the best pricing policy to adopt at this time is to establish a base price position in relation to a price leader. Typically, this role is naturally assumed by the business that provides a significant portion of supply and has taken the lead in establishing a particular market. Just as in production in which new businesses look to the experienced firms for expertise, so it

is with marketing. In this situation all producers need to collectively understand and accept a price norm set by a price leader, with due considerations for actual known costs. When this lead operation revises price either up or down, the remaining businesses should follow by instigating a similar price change.

It is important to emphasize that this policy is most relevant for pricing in major target markets in which no single firm has a distinct shipping and distribution advantage over another. But even for those producers who do have marginal shipping advantages and may be tempted to use the cost saving to enter the market on a price competition, it is likely to be far more advantageous and wiser to invest the higher profit into distinguishing the product through promotion and service (Chaston, 1983, Shaw, 1990). The intent is to broaden the customer reach and create stronger customer loyalty. These actions protect sales if and when another producer tries to enter the market at a lower price.

This pricing policy is advocated on the basis of two assumptions; the first is that there is sufficient information about how other producers, especially the largest regional producer, are pricing their T. derasa clams in various food markets. Information about the farmgate price, the price of a T. derasa sold directly to a buyer at the clam production facility, is particularly important. The second assumption is that the price leader has set price at a reasonable level that does not limit demand and consequently, does not encourage price competition.

In setting the price level for the National Aquaculture Center, all available marketing literature was reviewed to obtain information on pricing among T. derasa producers within the Pacific (Shang et. al., 1992; Stanton, 1990; Tisdell et. al., 1990; Iyechad, 1988). Based on the review and discussions with other individuals in the giant clam industry, the farmgate price for operations similar to the Center was found to be approximately $2.00 for a five inch clam. This figure provided a base price and was the most crucial piece of data to start with because the costs of shipping materials, air freight and distribution could be accurately calculated and added to this base price.

The other essential factors to account for in pricing were consumer acceptance of the product, and the level of promotional activity and service that is needed to stimulate demand. This would be assessed during the market test phase. After the evaluation of this test was completed, the final price for a clam in the Pohnpei market would be based primarily on the standards of other suppliers, and would also account for the actual costs of shipping, handling, air freight and distribution, and an initial understanding of demand within this market.

Promotion and Service

Promotion and service are two distinct but complementary areas of market development. Promotion refers to actions intended to persuade customers to buy. Service includes the actions that go into maintaining the relationship with the customer.

Promotion

The aim of promotional activity is to make potential customers aware of the desirable features of the product. Effective presentation of this information is done through a combination of advertisements and personal selling. In the foodservice market, emphasis is placed on personal selling to the restaurant owners and on advertisement to the consumer market.

For this project, personal selling was more formally organized into a sales presentation. The preliminary interviews with restaurant buyers held in the market assessment stage of this study constituted an in-person sales presentation. Within the format of the interview there was two way communication in which information on the product was given to the owners and the owners, in turn, gave immediate feedback to this information. This would ensure that the information was both relevant and effective in addressing the interests of the buyer.

Personal selling can be done either in person or by telephone. The results of the case study emphasize that although it is more expensive, face to face contact is extremely effective because it allows the chance to actually show the giant clams. For the interviews with restaurant owners, the Center staff brought live market size

T. derasa and displayed them at the beginning of the discussion. This format had effective sensory appeal and elicited immediate and positive reactions from the restaurant owners, served as a focal point for the interview, and precluded any possible misunderstanding about the characteristics of the product.

Feedback from restaurant owners not only indicated their interest in the clams but also revealed what other assurances they needed, especially about the stability and quality of the clams. Furthermore, restaurant owners said they would welcome any assistance in advertising the product to their customers and they suggested ways in which the Center staff could best provide that help. In particular, they mentioned that table tents would be a useful form of advertisement.

Their suggestions were translated into specific promotional actions. To alleviate concerns about the product, each restaurant owner was given a sample of ten clams. This type of promotion is often referred to as a special sales program and may also include price reductions, coupons and any other similar action that is expected to draw an immediate response from buyers. Although the response is usually short-lived, this is an effective method of introducing a new product. By providing owners with ten clams as a free sample, restaurant staff could gain experience and confidence with the product at no cost risk to them.

Advertising was another integral component of promotion in which restaurant owners welcomed assistance from the Center. In general, the restaurant menu alone is the most effective method of advertising a product, but details about any one particular food item are necessarily limited. Customers needed additional information about the clams to overcome the natural hesitations in trying an unfamiliar product. As suggested by the restaurant owners, the most inexpensive method for more comprehensive menuing is through table tents.

The information listed on the table tents was tailored as much as possible to the interests of the two target groups; tourists, especially Japanese, and the local population. A four by five inch table tent advertisement was designed in which three key points were made to create a particular image of the clams; first, the clams were described as "a local seafood delicacy." The notion of "local" was expected to appeal

to tourists who, according to restaurant owners, were especially interested in trying exotic local fare. Also, it was hoped that this statement would appeal to local residents because it capitalized on the promotional programs sponsored by government and public health organizations which encouraged local islanders to eat local foods. Second, the advertisement specified that the clams were "fresh" and "raised off the islands of Micronesia in crystal clear water." This indicated the source of the product and also fostered an image of distinction. The statement was also intended to alleviate any concerns about the quality of the clams and the conditions in which they were grown. Finally, the table tent was printed on two sides with a Japanese version printed on one side and an English version on the other.

Another promotional program was designed to stimulate demand by offering the clam shells as a souvenir to customers. The attraction and novelty of the shells would be a purchasing incentive and enhance the value of the product in the eyes of the customer. Restaurant owners were encouraged to keep a supply of clean giant clam shells on hand and to offer them to customers who ordered the appetizers. The Center also supplied restaurants with certificates stating that the shell was from a cultured giant clam so that tourists who wanted the shell could get it through customs. Giant clams are listed as an endangered species and the shells would be subject to confiscation by custom officials in certain countries, including Japan and the U.S., without some verification that the shell was not from wild stock.

Service

While promotion is intended to get the interest of customers, service is intended to keep customers interested. Just as in designing a promotion strategy, the service policy needed to be tailored to the needs of the buyers. To effectively match specific services with the particular needs of the buyers in the Pohnpei market, restaurant owners were asked what services they would most like to have. These were: 1) assurance of product quality and delivery, 2) help in finding a holding location for the clams, and 3) guidance in preparation and handling.

To assure product quality, the standards for consistent sizing and scrubbing of the clams were emphasized to the Center staff members involved in selecting and

shipping the clams. In addition, the Center established a policy that if clams died or otherwise seemed unhealthy within 24 hours of delivery to the restaurant, they would be replaced. For delivery assurance, the role of the distributor was explained and restaurant owners were told they could expect consistent weekly contact from him; the distributor would call for orders on Wednesday mornings and deliver the clams on Thursday afternoon.

Next, a staff member from the Center visited the restaurants to help owners choose a suitable holding container and area. During this visit, the staff member distributed the set of instructions on clam preparation and complemented this guide with an actual demonstration on how to shuck and prepare the clams. This reduced the chance of error in following the written instructions.

A final service area concerned the billing schedules. For the most part, restaurant owners reported they usually paid for goods upon delivery. However, since these were well established businesses with good reputations, the Center offered them the option of paying within 30 days of delivery. Under this options, restaurants would receive an invoice with each weekly shipment and a monthly balance statement.

From the perspective of the Center these service actions were considered to be an investment toward ensuring the success and confidence of the new customers in handling the new product. The rationale underlying the service policy is that costs of providing any particular service must be weighed against the expected benefits. The services selected by the Center represent what was believed to be a balance between these costs and benefits.

Evaluation

Throughout the development process, the preliminary research and small-scale exposure of the clams to restaurant owners and potential restaurant customers was helpful in formulating the first draft of the plan as described in this report. The final step was to evaluate the entire marketing plan and assess its viability. This was done on the basis of a market test designed to see if all the components of the plan taken together, the product, the shipping and handling, the distribution system, the promotion and service, could produce the volume necessary to establish a reliable

market. It would also identify weaknesses in the plan and suggest ways in which it could be strengthened.

The three particular objectives of the market test were: 1) to assess consumer acceptance of giant clams in the Pohnpei foodservice market; 2) to assess the functioning of the product selection and packing protocol and the distribution system and; 3) to assess the satisfaction of the restaurant owners. This objectives were met by conducting a survey of customers who tried the clams during the test, monitoring the performance of the distribution system throughout the test period, and through personal interviews with owners regarding their experiences and opinions on the product and performance of all related marketing activities.

Three of the four restaurants who participated in the initial market evaluation were asked to take part in a four week market test. All enthusiastically agreed to participate. At the suggestion of the restaurant owners, the test was scheduled for a four week period during late February through mid-March, 1993. This corresponded to an off-peak period when it would be easier for the restaurant owners and their staff to accommodate the additional time and effort in handling a new product and conducting a consumer survey.

The survey consisted of three questions. The first asked respondents to rate the clams using a five point scale in which a score of 1 was "Poor" and 5 was "Excellent." Second, consumers were asked if they would ever order giant clams again. If they answered "no" they were asked to explain why not. The third question asked respondents to specify their residence as either "Pohnpei" or "Visitor". The survey was written in both English and Japanese and printed on the reverse side of the table tents.

During each of the four weeks of the test period, every restaurant received up to fifty live clams at no cost. The owners were free to choose any preparation method and to set their own price, although they were asked to consider pricing based on a wholesale price of approximately $2.00. In return, restaurant owners kept track of the number of clams served, the number of customers who ordered clams, and the menu price.

Throughout the test period, all restaurants chose to menu the clams as sashimi appetizers. Clams were sliced and presented in the clam shells with a garnish and dipping sauce. The menu prices varied considerably between establishments, ranging from a low of about $2.50 to a high of $5.00. Customers were allowed to keep the shell if they requested.

The results of the consumer survey are summarized here from a comprehensive report (Peavey and Riley, 1993). The survey had an overall response rate of 69%, with 134 customers completing useable returns. Ratings were predominantly positive, with "very good" as the most common response. Furthermore, nearly 85% said they would order giant clams again. More detailed analysis indicated that the local residents tended to give higher ratings and were more likely to say they would order clams again, in comparison to the visitors.

Just over 23% of the respondents listed a complaint. The most common problem was that the portion size was too small. Understandably, customers indicated they were disappointed in the size of the appetizer because of their conception of how big a "giant clam" serving would be. Other complaints were sporadic and varied.

The general conclusions from this information is that the giant clams achieved a high level of consumer acceptance in the Pohnpei market, especially among the local residents. This was an important finding because it suggested that rather than having to rely completely on tourist demand and its corresponding high and low fluctuations, there was a significant potential for relying on local residents to provide some minimal level of demand that could sustain the functioning of the market throughout low tourist periods.

In addition to an assessment of consumer satisfaction, the market test was used to appraise the functioning of the distribution system. The experiences during the test period of the distribution system emphasized the number of issues involved in ensuring that the system performs all the essential functions outlined in the plan. It also revealed the consequences that can occur if the distribution roles have not been adequately defined or if the intermediaries are incapable of executing the distribution responsibilities.

The first problem occurred with the initial shipment of clams in Test Week 1. Restaurants had been contacted by telephone to tell them the exact day they could expect delivery of their first 20 clams and they had been given an information packet containing the handling and storing information. Nevertheless, at two of the three restaurants, no one from the restaurant staff was available to handle the delivery of the clams. Since the agreed upon role of the distributor was simply to deliver the clams, the boxes were left with various other employees at the locations. The results were disastrous. At one restaurant, the shipment was opened in the late afternoon of the delivery. After opening the box, no one transferred the clams to seawater. Approximately 10 of the twenty clams died by the following day. At the other restaurant, the shipment was not opened until two days after delivery and as a result, all 20 of the clams were dead when the box was finally opened. However, at the third restaurant where one of the kitchen staff had accepted the delivery and placed the clams in the storing tank with water, all clams remained healthy throughout the week.

This outcome indicated that the Center should assume greater involvement in the distribution process, at least during this start up phase. For the second shipment during Test Week 2, a Center staff member personally delivered the clams to ensure that the handling and storing instructions were understood and would be followed for the remainder of the test. The staff member also brought aerators to put into the holding containers at each restaurant so that the clams could be held longer in the same seawater. This would make the holding situation more convenient for the staff.

The next mishap occurred during Test week 3 and again related specifically to the performance of the distribution system. During that week, the distributor had a prior commitment associated with his primary occupation and had informed the Center staff that someone would need to fill in for one particular shipment. A person in Pohnpei who was enthusiastic about the project agreed to handle the delivery.

To simplify the arrangements, the regular distributor set up a holding tank at his house in which he kept fifty clams to be used for delivery during the week of his absence. The substitute would not have to wait for the shipment to arrive at the airport, but could simply gather up the clams from the tank and deliver them. The

afternoon prior to the scheduled delivery day, one of the Center staff contacted the substitute to remind him of the delivery and to tell him how many clams should be delivered to each restaurant. Late the next day, the Center received calls from the restaurant owners asking why they had not received their clams. Repeated attempts to contact the substitute were unsuccessful. Two days later, the restaurants had still not received delivery and the substitute could not be located.

This situation necessitated another trip to Pohnpei so that the Center could restore the functioning of the distribution system. A check of the holding tank showed that all 50 of the clams were either missing or had died. New shipments of clams were sent to Pohnpei and delivered personally by the Center employee.

The regular distributor resumed his delivery service for the final test week and no other episodes took place. Together, the four restaurants sold just over 300 giant clams during the four week period. But because of the storage and distribution problems during week 1 and week 3, demand for clams had been constrained by an insufficient supply.

Interviews with restaurant owners at the conclusion of the market test constituted the final component of the evaluation. Their comments confirmed the impact of the malfunctioning distribution system on the level of their satisfaction with the marketing plan. However, the immediate actions from the Center in addressing the problems had been reassuring to the restaurant owners and helped foster confidence in the responsiveness and capabilities of the Center.

Product handling was only a problem during the first week and overall their level of satisfaction in this area was high. Once they established a suitable holding location for the clams and began using the aerators for circulating the water, the inconvenience of handling the new product was reduced. After a few trials, the chefs were able to quickly clean and prepare the clams for sashimi.

One owner reported a significant finding about the effect that the clam appetizers had on total sales. His initial concern had been that the clams would cannibalize from other more profitable menu items. However, after the test he concluded that customers who ordered clams actually spent more per check. This

implied that the customers were not foregoing other items on the menu, such as dessert or drinks, to make up for the expense of the appetizer. It seemed that the clam appetizers induced customers to spend more money at the restaurant. This was an important point that would be especially interesting to any restaurant owner.

The most common complaint among owners was the same as their customers; the serving sizes were too small. Another aggravation was that the sizing was too variable and inconsistent. In several cases, the owners had to use two clams to make a presentable single serving of sashimi. Because of these experiences, they revised their preference figures from the previous four to five inch clam to a five to six inch clam and asked for greater attention to consistent sizing.

At the completion of the test market, Center staff were able to calculate a delivered price of the clams. The added costs of packing, shipping and distribution raised the delivered price of the clam to $2.50. Using the sales data collected during the test, potential demand was also estimated. The figure of 300 clams sold during the four week period offered a conservative estimate of potential volume due to the distribution and handling problems.

With consideration to the revised clam price, the various menu prices used during the market test, and qualifications of the sales volume, an estimate of future demand was estimated at 25 clams per week per restaurant. Owners were asked to react to these figures. All three indicated that the $2.50 price of the delivered clam pushed the limit of what they were prepared to pay. One owner felt the price was simply too high and that to achieve their desired margin, a single clam appetizer would have to be menued at $7.50. This was too high for their menu. The top price they were willing to pay was $2.00.

However, two of the owners said they wanted to begin buying the clams, but would have to raise their menu prices; one said he would menu the appetizer at $4.00 and would probably order 20 to 25 clams at a time. The other owner mentioned a price of $4.50 and a weekly order of about 15 clams each week. Both pointed out that their orders would increase and perhaps double during their busy tourist times in May, August and September, and possibly January.

A weekly order of 35 to 40 clams would not even meet the minimum shipping charge. But with these two businesses as regular customers, more marketing experience could be gained and a broader market could be developed. As a result, the market test not only served as a vehicle for designing, testing and evaluating a market plan, but also was successful in obtaining the first customers for the FSM National Aquaculture Center.

RECOMMENDATIONS

Recommendations were formulated for each component of the marketing plan. They include appraisals of actions encompassed in the plan and suggestions for revisions.

Market Analysis

Continue to recruit new buyers in the Pohnpei foodservice Market. Foodservice should continue to be the central focus of marketing efforts. Using the same criteria as in the market analysis for this study, other restaurants in Pohnpei should now be approached. But the intent should not be to simply to sell clams to as many restaurants as possible. It is crucial to carefully assess the ability of any new buyers to handle and prepare a unique, perishable product such as giant clams. If one restaurant serves clams in a poor presentation or clams that have deteriorated as a result of improper handling, consumer satisfaction will be affected and as a result, the overall demand for clams can be seriously damaged. Even worse, it is possible that customers could become ill from eating clams that have not been properly handled or prepared. Obviously, the subsequent impact on demand would be devastating.

Introduce clams in the local Pohnpei seafood market. In addition to foodservice, the Center should also investigate the potential for selling clams in the local seafood market in Pohnpei. Since foodservice is limited in this FSM state, local demand can be tapped by selling in other food markets. This local demand has the potential for complementing the foodservice component and can broaden the base upon which the business develops.

The Center should initiate product development along with market development. This project focused on giant clams as food and was not designed to address the issues involved in establishing giant clams in aquarium markets or in markets for clam shell handicrafts. The Center will need to extend marketing efforts into these arenas because reliance on a single product in a single market is extremely risky. However, even in the foodservice market alone there are significant opportunities for developing a range of products and creating a product line.

As the restaurant owners gain greater familiarity with using giant clams in their operations, one possibility for product development is to introduce other species of similar quality that have been verified through sensory testing. H. hippopus is one species that has been tested and should be considered for introduction to expand the product line (Peavey and Riley, 1993).

Further work should be directed toward devising a protocol for maintaining the quality of shucked fresh or frozen giant clams. A high quality frozen clam could be positioned as a convenience item for foodservice and would greatly reduce shipping costs.

The product mix can also be diversified by creating a full line of giant clam shell craftware specifically designed for foodservice use. Examples include wasabi dishes, salad bowls and candle holders. These items are likely to appeal to foodservice operators and complement their use of the clams.

Work toward developing export capabilities. On a broader scale, foodservice markets with sufficient volume are limited within the FSM region. Consequently it will be essential to move into exporting. The principles for export marketing are the same as for marketing within the local region. The difference lies in the complexity, stability, intensified competition, and costs. The Center will need to accurately assess how well it can serve a larger export market by carefully evaluating its ability to supply the sizes, quality and volumes required. Advantages may offset the difficulties, but it will require commitment, greater expertise and sophistication. Experience and time in the local market will help develop these traits.

Handling and Distribution

Restaurants should be consistently supplied with T. derasa clams of approximately six inches, with five inches as the absolute minimum. During the market test, clams were sized inconsistently and generally too small. This led to dissatisfaction among the restaurant owners and their customers. A check system should be instigated during packing in which one staff member sizes and selects clams using a gauge and a second staff member verifies the sizing by measuring the clams again. Clam selections for shipping should be done by the same staff members each week because over time they will become familiar with the correct size and are likely to select and clean the clams using consistent appearance standards.

Constantly monitor the performance of the distribution system. The biggest obstacle to successful development of the local market is the instability of the distribution system. In this test, the shortcomings of the system emphasized the essential characteristics of the people or agencies hired to perform different roles in the system; reliability and commitment. Understandably, it is extremely difficult to find the right people, set up the requisite structure and maintain efficient performance within a system that is only used part-time.

The roles of the intermediaries as agreed to in this situation are not necessarily the most desirable. They reflect the cautious nature of those involved in introducing or handling a new product within a new market. As the market expands and becomes more predictable the role of the distributor may need to be renegotiated.

The importance of ensuring the adequate performance of the distribution cannot be overstated, especially in dealing with a product as perishable as giant clams. During the test market, approximately 75 clams were lost because of distribution and handling mistakes. In the longer run, an unreliable distribution system leads to even more significant losses in terms of lost customers who have become frustrated with delivery irregularities and delays. Obviously, this has far reaching consequences for the successful development of a market. During the start up stage which can last for months before distribution becomes routine, nothing should be taken for granted. At the very least, the Center should be diligent in seeing

to it that all intermediaries involved in the distribution complete their task. At the other extreme, the Center should continue to be ready to step in and assume the responsibility of delivering product if necessary.

This situation would be vastly different in larger markets in which distribution systems are established and include a number of other possible intermediaries, such as freight forwarders who assume major responsibilities in the flow of physical product without taking title to it, dealers and foodservice distributors, who assume ownership of the product and take on a large share of promotion activities and brokers, who play a major role in promotion, but do not take ownership of the product. A central step in developing an export market plan will be to identify the various intermediaries already established within the Pacific region and to understand the responsibilities they typically assume.

Pricing

Price on the basis of a price leader or an established norm within the region. Concerted efforts are needed to monitor and update all available price information and identify regional norms for pricing giant clams in various food markets. This can be done by staying in regular contact with other clam producers, attending trade meetings, reading trade publications and newsletters that cover activities in aquaculture and seafood marketing.

Use the regional price norm as a guideline and add on any additional shipping and handling costs associated with particular markets. As the general rule, the Center should price competitively on this basis, but should not try to "buy" its way into a market by undercutting the established norm. In this study, a farmgate price norm for the five to six inch T derasa was found to be approximately $2.00. Adding on the shipping and delivery costs boosted the price to $2.50 per clam in the Pohnpei market.

Investigate the possibility of forming a marketing cooperative or group. The Center should initiate discussion with other giant clam facilities to explore the desirability of starting a marketing cooperative. There are several basic principles upon which these groups usually operate; members must commit a certain percentage

of their production to the group for which the cooperative establishes a price. The group establishes standards of quality and has the power to reject unsatisfactory products from its members. Production schedules are worked out by the group and members must adhere to them to the best of their ability.

It takes considerable time and negotiations to set up a group but it offers the opportunity for obtaining stability in the market and diminishes the risk of investing in market development. An effective marketing cooperative reduces the potential for price competition, a situation which would very likely lead to chaos in a developing market because committed group members must abide by the group price. Furthermore, they assure a certain level of quality which also promotes stability in new markets. A complete feasibility study should be conducted to assess the possible benefits and disadvantages of a cooperative, to outline conventional methods of organization, structure and operational guidelines, and to investigate interest among Pacific Giant Clam suppliers.

Until this option is pursued, the policy of following the price established by a price leader for larger export markets seems to be the optimal choice and is likely to foster the vitality of the industry as a whole in this region. This is assuming that the product itself is fairly homogenous, and that the price leader is not setting a price which excessively constrains demand.

Promotion

Maintain essential supplies and establish guidelines for personal selling. Promotion can quickly become very expensive for a small business, but the results of the test suggest how fundamental promotion efforts can be undertaken in both personal selling and advertising. Personal selling is directed toward the restaurant buyers. The fundamental tools for personal selling include letterhead paper, envelopes and business cards. The Center should also consider investing in a brochure that describes the business and its products. A brochure is an effective method of communication and fosters an image of professionalism. Design of this brochure should be done in collaboration with an advertising agency or printer who has experience in this field.

Similar to the course of actions followed for this project, approaches to new prospects should be made first by sending a brief letter that describes the production facility and the giant clams. The closing of the letter should state a particular day on which a staff member will call the buyer. This telephone call can be used to explore the buyer's interest and to answer any questions.

A decision has to be made between conducting sales presentations strictly through telephone or by in person contact. Face to face contact is favored whenever possible because it is more persuasive. Since most buyers are unfamiliar with giant clams, live clams should be brought to the interviews; the unique characteristics of the clams elicit immediate, positive response from people. Photographs do not adequately substitute for the actual product.

Whether it is conducted by phone or in person, the sales presentation should continue to be perceived as an interview or discussion with prospective buyers. The results of the consumer survey and restaurant owner interviews can be used to verify consumer and buyer satisfaction with the product. These points are included in a sample sales presentation/restaurant interview script included in Appendix B.

At the conclusion of the sales presentation, a sample of 6 to 10 clams should be offered to those prospects who express a sincere interest in menuing the clams. Although the samples were provided free for this project, they can be offered to new buyers at an introductory reduced price, or at cost of shipping and delivery only.

A final point should be considered in establishing guidelines for personal selling. Within this region, a high proportion of the restaurants that are excellent prospects for menuing giant clams are operated by Japanese business people. Those involved in selling giant clams should have some rudimentary abilities in speaking Japanese. Even if those skills encompass nothing more sophisticated than being able to convey simple greetings in Japanese, the effort is likely to go far in establishing a positive rapport and developing a desirable image of the Center.

Encourage use of giant clams in other preparations. Promotional efforts should also be directed toward encouraging restaurant owners to use the clams in preparations besides sashimi. In this case study, a collection of recipes was given to

the owners. To expand on this action and give it greater impact, these recipes should include photographs of the preparations. Visual presentations are likely to have far greater influence than written recipes alone.

Table tents should be used for advertising to restaurant customers. The results of this study verify that table tents are an important medium for communicating directly with the restaurant customers. Moreover, feedback from consumers helped identify specific revisions that would enhance the effectiveness of the message. Table tents should be redesigned so that the clams are identified as "baby" giant clams. This would reduce the risk of creating misconceptions among customers regarding the portion size of the appetizer. Information on the tent should also clarify that the clams are cultured and are grown and harvested locally off the islands of Micronesia. This would appease customer concerns about depleting a natural stock of wild clams. Also, to add to the perceived value of the product, the table tents should state that customers are invited to keep the shell as a souvenir. A table tent that incorporates these revisions is contained in the Information Packet in Appendix A .

Restaurants will need to keep a supply of Certificate of Origin forms to give to tourists who want to keep the shell. Without these forms, customs officials in certain countries, including Japan and U.S., would confiscate the shell since giant clams are listed as an endangered species. A copy of the Certificate of Origin used in this project is also included in Appendix A.

In devising additional promotional activities, the role of the waitstaff should be recognized as a vital method of promotion. Because of their direct contact with customers, the waitstaff are the primary conduits of customer information and can have the greatest influence on the customer's purchasing decision. The Center should extend efforts to educating the waitstaff about giant clams and how they are served so that they will be able to pass this information on to their customers.

In addition, emphasis on public relations as a promotional strategy can go far in enhancing the image of both the giant clams and the production facility. Donating clams for local celebrations, inviting the public to visit the facility or helping to

sponsor public events are three ways in which the Center can creatively integrate public relations into its promotion plan.

Service

Identify basic services and consistently provide them. The service component of the plan covers the broad range of coordinated activities designed to retain customers, increase sales from existing customers and generate sales from new customers. The marketing plan for this study included fundamental services that are essential to any business operation and more elaborate optional services that were intended to facilitate use of the giant clams and buyer satisfaction.

At the most basic level, routine services such as order taking, delivery and billing need to be done on schedule. Periodic courtesy visits or telephone calls should be made to the restaurant owners to foster a friendly relationship and to encourage buyers to discuss their problems. Equally important, intermittent contact should be maintained with restaurant owners who have been introduced to the giant clams but have chosen not to buy the product. The situation facing these buyers may change considerably over time. By staying informed on their status, the seller may be able to present the clams more effectively so that they are perceived as meeting particular needs of the potential buyer.

Service basics should also include having a back up telephone answering machine. The answering machine should contain a message stating when someone will return the call and should allow callers to talk as long as they need to. In a giant clam production facility, employees are often working outside of the office. As a result, there is a risk of missing telephone calls. This is detrimental from a service perspective because customers can be easily frustrated at not getting any answer to their calls, especially if they are calling with a problem. The unanswered telephone also detracts from the image of stability and reliability.

Similarly, the fax machine is now a standard feature in business. Because of occasional delays in mail service within this region, a fax machine is also highly recommended. It can alleviate the lags inherent in mail contact and offers a way of communicating with people in other time zones when a telephone call is inconvenient.

Provide additional key services to develop a competitive edge in the market. More extensive services will help distinguish the Center as a reliable supplier of giant clams. Product handling in particular, will require greater emphasis on services. An information packet containing instructions for storing, handling and preparing giant clams should be distributed to new buyers. However, this packet should be treated as a reference guide since complete reliance on written instructions is risky, especially with a product as perishable as giant clams; reading instructions is time consuming for restaurant staff and, as a result, many neglect to do it. Moreover, in this particular market, the majority of restaurant owners and their staff do not speak English as their first language and abilities to communicate in English are extremely varied.

To address these problems, the Center should conduct clam cleaning and preparation demonstrations to new customers whenever possible. If the costs of doing this in person are prohibitive, the Center should consider making a video film of this demonstration which can be loaned to new customers. The video can be as effective as the personal demonstration in overcoming many of the frustrations in trying to communicate in a variety of languages. The photograph series of clam cleaning and preparation instructions developed in the case study should also be given to new customers to use as a reference guide.

The Center should arrange to help new customers select a location for holding the clams at their restaurants. The Center could negotiate with the distributor to perform this service, or could arrange to have a staff member visit the restaurant. To those customers who seem committed to buying clams after an initial trial period, the Center should either provide an aerator to use in the holding tank or offer these to restaurants at cost.

CONCLUSIONS

The development of an appropriate marketing plan is essential in successful market development. It provides the guiding basis for the entire collection of marketing activities involved in moving the product from the point of production to the point of consumption. This case study involved devising strategies to meet the

objectives of the marketing plan, identifying and coordinating specific actions associated with the strategies, and assessing the impact of these actions.

Mistakes are inherent to any marketing plan. This emphasizes the need for systematic evaluation of the plan, as was done in this study, to understand why the problems occurred and to revise the plan accordingly. These improvements tailor the plan to fit the actual market situation. Since markets are continually changing, it is essential to regularly monitor buyer satisfaction, consumer acceptance and the performance of the distribution system. A formal evaluation should be conducted every three months during the first year of market entry.

It is difficult to standardize a marketing plan that will fit the needs of all giant clam suppliers in the Pacific. Nevertheless, this guide should be relevant to suppliers throughout this region because they are facing similar marketing conditions. Their capabilities for market development will be strengthened by understanding the need for a market plan and the central aspects of developing and implementing their own plan.

REFERENCES

Braley, R.D. "Improved Method for Shipping Tridacna gigas seed." Aquaculture, Elsevier Science Publishers, B.V., Amsterdam, 102 193-199.

Buell, Victor. 1986. "Strategic Market Planning," in Handbook of Modern Marketing, Victor Buell, ed, Mc Graw-Hill Co. New York, 45.3-45.13

Chaston, Ian. 1983. Marketing in Fisheries and Aquaculture, Fishing News Books Ltd, Farnham, Surrey, England.

Dawson, P.F. 1986. "Report on a Study of the Market for Giant Clam Products in Taiwan, Japan, Hong Kong and Singapore." FFA report #86/37, South Pacific Forum Agency, Honiara, Solomon Islands.

Food Institute. 1990. Food Retailing Review. Food Institute Information and Research Center, Fairlawn, New Jersey.

Gibson, Lawrence. 1986. "Researching and Market Testing New Products." in Handbook of Modern Marketing, Victor Buell, ed, Mc Graw-Hill Co. New York, 42.1 - 42.10.

Iyechad, Gwenda. 1988. "Clams for All Seasons," in Star-Bulletin and Advertiser, Honolulu, Gannett Pacific Corp.

Office of Planning and Statistics, Federated States of Micronesia. 1991. "Visitor Arrival Statistics." Division of Tourism, Dept. of Conservation, Pohnpei, FSM.

Peavey, S. and John Riley. 1992. "Exploring the Potential for Giant Clams in the Local Foodservice Market." Report No. 92-01, National Aquaculture Center, Kosrae, FSM.

Peavey, S. and John Riley, May 1993. " Consumer Acceptance of Giant Clams in the Local Foodservice Industry." Report No. 93-03, National Aquaculture Center, Kosrae, FSM.

Peavey, S. and John Riley, March 1993. "Sensory Evaluation of Fresh and Frozen Giant Clams." Report No. 93-02, National Aquaculture Center, Kosrae, FSM.

Peavey, S. and John Riley, July 1993. "Sensory Evaluation of Three Giant Clam Species: Tridacna gigas, Tridacna derasa and Hippopus hippopus. Report #93-05, National Aquaculture Center, Kosrae, FSM.

Shang, Yung, Clem Tisdell and PingSun Leung. 1990. "Report on a Market Survey of Giant Clam Products in Selected Countries." Center for Tropical and Subtropical Aquaculture. U.S.D.A., Publication #107

Shang, Yung C., PingSun Leung, John Brown and Clem Tisdell. 1992. "Test Marketing of Giant Clams as Seafood and as Aquarium Specimens in Selected Markets." Center for Tropical and Subtropical Aquaculture, U.S.D.A. Publication #110.

Shaw, Susan. 1990. Marketing. A Practical Guide for Fish Farmers. Fishing News Books Ltd, Farnham, Surrey, England.

Stanton, John. 1990. "Evaluation of International Trade Statistics on Giant Clams and Related Products and the Market for Giant Clam Meat." Research Reports in the Economics of Giant Clam Mariculture, No. 9. University of Queensland, Department of Economics.

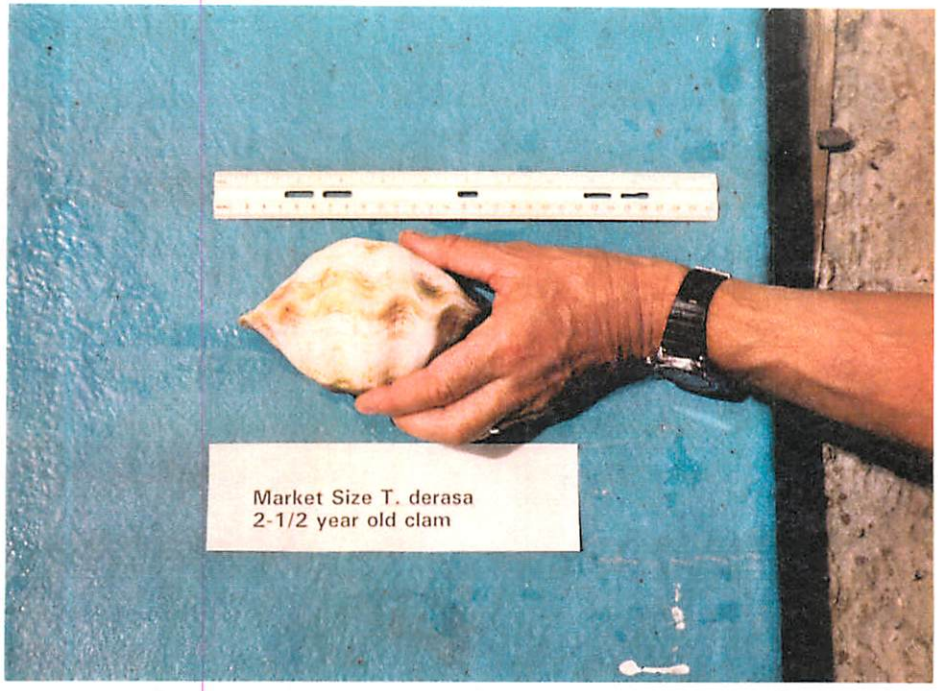
Tisdell, Clem. 1989 "Pacific Giant Clams and Their Products: An Overview of Demand and Supply Factors." Research Reports in the Economics of Giant Clam Mariculture, No.5 University of Queensland, Department of Economics.

Tisdell, C., J.S. Lucas, and W.R. Thomas. 1990. "An analysis of the Cost of Producing Giant Clam Seed in Australia." Research Reports in the Economics of Giant Clam Mariculture, University of Queensland, Department of Economics.

Tisdell, C. 1992. "Interest of Asian Restaurants in Queensland in Using Giant Clam Meat in Their Cuisine and Their Knowledge of it." Research Reports in the Economics of Giant Clam Mariculture, No. 35. University of Queensland, Department of Economics.

Tisdell C., and Y. Kuronuma. 1992. "Interest of Japanese Restaurants in Brisbane in Using Giant Clam Meat in Their Cuisine and Their Knowledge of it." Research Reports in the Economics of Giant Clam Mariculture, No. 37. University of Queensland, Department of Economics.

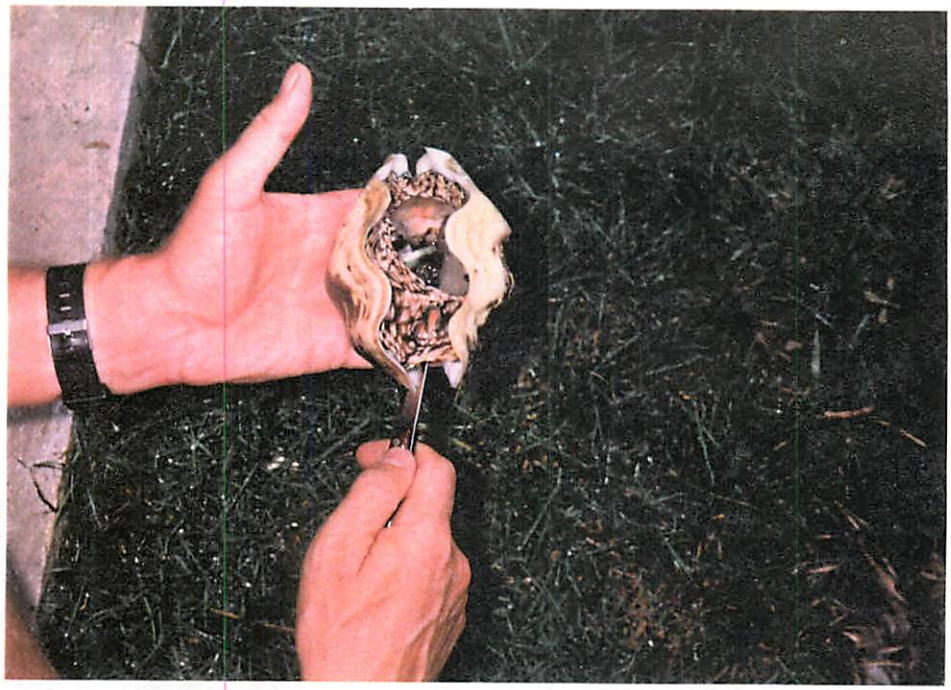
Tisdell, C. and Y. Juronuma, 1993. "Giant Clams in Japanese Cuisine Brisbane Trials and the use of Giant Clams in the Ryukyus." Research Reports in the Economics of Giant Clam Mariculture, No. 39. University of Queensland, Department of Economics.



1. Tridacna derasa giant clam is the most popular species for sashimi. The preferred size for sashimi clams is approximately 6 inches (15 cm). This is when the texture and flavor of the meat is at its best. Giant clams reach this size when they are between 2 and 3 years old.

2. For preparation, first insert a knife through the byssal opening located at the back of the shell.

3. Move the blade of the knife toward you to cut the muscle. You may have to move the blade back and forth a few times, but do not try to pry the shell open by wiggling the knife up and down.



4. Once you cut the muscle, the shell will immediately open up.

5. Loosen the clam meats from both sides of the shell.

6. Now separate the edible portions of the clam from the viscera. The largest edible section is the mantle, the multicolored meat. Separate this piece from the other parts.

7. Next locate the adductor muscle portions. This is the firm white meat that resembles a scallop. Usually you will find two pieces of adductor muscle. Remove the membrane around the muscle meats.

8. After separating the muscle pieces and mantle, all that remains is the viscera section containing the kidney and stomach. This should be discarded.

9. Rinse the mantle and muscle meats. Chop the pieces for sashimi preparation.

10. Put the chopped meats into one half of the shell. Make a dipping sauce, such as soy and lime, and place this in the other shell half. Add wasabi, lime slices, shredded cucumber or other garnish as desired.

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APPENDIX B. Sales Presentation/Buyer Interview Outline

Description of other restaurant experiences with giant clams: Our experience is based on our test market in Pohnpei. These restaurants experimented with preparation and menuing and found that for them, giant clams worked best a sashimi appetizer, one clam per serving. They chopped the clam meats and served it with an accompanying sauce and presented it to the customer in the shell. They experimented with price and are now consistently selling the appetizers for $4.00 to $4.50.

These restaurant owners have reported to us that the clam appetizers do not cannibalize from other menu items. In other words, customers who order clam do not seem to compensate by cutting back on drinks or desserts, or by ordering a less expensive entree. Rather, they spend more per meal overall.

I. Familiarity with Giant Clams

1. Have you ever tried Giant Clams?
   a) If so, where and how?
   b) Have you actually served them at your restaurant?
   c) How was your experience with them?

   If no, what do you know about Giant Clams?
2. Tell me about your customers (Predominantly tourist? From where? Popular menu items? Interest in exotic food?
3. How do you think your customers would respond to sashimi giant clams? Do you think you'd be inclined to serve giant clams as an appetizer or entree?
4. What about other preparations? Would you be interested in other recipes using giant clams?

II. Product Preferences and service needs

1. What is your general impression of this product?
2. How about the size; what size do you prefer and why?
3. Presently we are marketing the product as whole fresh clams in the shell. Would you have any interest in processed giant clams, such as; (a)shucked fresh clams on ice, meat only; (b) frozen clam meat (c) other suggestions?
4. What are your usual arrangements for product deliveries? How often would you expect to have deliveries?
5. What concerns do you have about buying and serving giant clams?
6. What type of information or service would you like to have?

III. Price and Demand estimates

1. What is your seating capacity?
2. When are your busy times/peak tourist times of the year?
3. What price would you expect to pay for this product, including shipping and delivery?
4. (For a sashimi appetizer) At what price would you menu the clams?
5. Can you give a general idea of how many clams you might serve during a busy week? During a slow week?

APPENDIX C: MARKET ANALYSIS IN CHUUK

The first test market for giant clams was conducted in Pohnpei partly because of its close proximity to Kosrae and subsequent ease of air shipping, and partly because of the strong potential demand corresponding to the large number of restaurants catering to Japanese tourist. In September 1993 a second test market was conducted in Chuuk and as in Pohnpei the local foodservice market was targeted.

A listing of popular restaurants in Chuuk was obtained with the assistance of a local travel agent. Three restaurants were selected based on the advice of frequent visitors to Chuuk. The owners were sent an introductory letter with information on the project and told that staff from the Center would be coming to Chuuk on business and would contact them for an interview.

The first interview in Chuuk was held with the general manager of a major resort hotel. Using the same interview protocol as in Pohnpei, the manager was shown a live market size clam, asked about his familiarity with product and given information on pricing, supply and distribution, storage and preparation. The manager was enthusiastic about trying them and felt that the clams would complement the menu. He indicated the price was not quite in-line with his policy; ideally, he aimed for a 300% markup on the "center of the plate" items, but he was willing to try menuing the clams at a price of about $5.00 for a sashimi appetizer, assuming a wholesale price of $2.50, delivered. He accepted the offer of a test shipment of 10 clams at no cost, together with a demonstration of preparation for his kitchen staff.

The Center Staff next visited a Japanese restaurant that catered to both tourists and locals. A study of the menu showed a high percentage of seafood items and a price range compatible with a $4.00 to $5.00 appetizer. Moreover, the restaurant had an elaborate display of working aquariums throughout the restaurant. This would have provided an ideal medium for featuring giant clams.

Attempts to communicate with the owner were ineffective because he spoke no English at all. The head cook, a Trukese who spoke some English, was approached for assistance but he indicated he was not interested. The next day, the Center staff

members returned to the restaurant with a live clam to show to the owner. They also gave him a copy of the table tent that was written in Japanese. But it was obvious that he still did not understand the purpose of the visit and was frustrated with the situation.

Visits were made to virtually every other restaurant in Weno to evaluate their menus and prices. It was apparent that giant clam sashimi appetizers menued at a price of $4.00 to $5.00 would probably not be appropriate for these establishments, since the highest priced entree on the menus was less than $6.00. These contacts were not pursued.

However, the Center staff were urged by several government employees to visit one final restaurant that was a popular local establishment. The owner and his manager agreed to an interview. They were familiar with giant clams and the manager said he had prepared and eaten them. After they were shown a clam and given the sales information, they felt that the price was too high but said they would be interested in testing a sample of clams on some of their customers.

Two weeks later, a second trip was made to Chuuk to distribute the samples at the two restaurants. The Center staff delivered 10 clams to the local restaurant along with an information packet on storing and handling the clams and 6 table tents. The manager declined the offer of a cleaning demonstration, claiming he knew how to clean and prepare them.

Another 15 clams were delivered to the hotel restaurant. One of the Center staff gave a preparation demonstration to the kitchen crew and helped select a suitable holding location for the clams in a well lit portion at the back of the kitchen. The manager indicated that changing the water was not a problem since the hotel was situated on the beach.

Follow up calls were made to the restaurants the next week. At the local restaurant the manager reported that the clams were popular but as he expected, too expensive for their menu. However, the general manager at the hotel restaurant was enthusiastic about the reaction from his customers to the sashimi clams and felt that the menu price was acceptable. He had some concerns about keeping the clams in

storage between weekly deliveries but was reassured after hearing about the experience of restaurant owners in Pohnpei. His biggest hesitation at that time was that he had hired a new chef who would be arriving sometime within the next few weeks. He wanted to give the chef time to become acquainted with the operations before asking him to try a new product such as giant clams. He indicated he would get in touch at a later date.

Although air shipments are feasible from Kosrae to Chuuk, the advantage of the Chuuk market was that a test clam farm had been established in Chuuk during the previous year in which a large proportion of clams had reached the desirable market size. It was hoped that once the market was established, seed clams would be sent from the Center, raised for one year at the ocean grow-out site, then brought in for marketing. The farmer was contacted for an interview to explore his interest. Although enthusiastic at first, the farmer lost interest when he learned that only one restaurant wanted to order clams and that the likely size of that order would be about 25 clams per week. The Center decided to offer the clams air shipped, but with no distribution system in place, the hotel manager would have to pick up the delivery himself. Added to this, costs of air freight would be prohibitive without a minimum order requirement.

On this basis it seems that the Chuuk foodservice industry does not represent a viable market for giant clams at this time. However, new hotels and restaurants are being constructed and planned. It is recommended that the situation in Chuuk be reviewed again during late 1994. Results from direct sales to locals in Kosrae and Pohnpei should also be monitored with the possibility of similar, seafood market selling in Chuuk to supplement the foodservice market.

Final Report

Pacific Aquaculture Association

Market Promotion for the Development of Cultured Marine Shrimp on Guam

By: David J. Landkamer, William J. FitzGerald, Jr., and Michael Bauerlein,

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DEPARTMENT OF COMMERCE

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PACIFIC AQUACULTURE ASSOCIATION

MARKET PROMOTION FOR THE DEVELOPMENT OF CULTURED MARINE SHRIMP ON GUAM

FINAL REPORT

Prepared by

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March 1994

Guam Aquaculture Development and Training Center
Department of Commerce
Government of Guam

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PACIFIC AQUACULTURE ASSOCIATION
PROJECT REPORT

FINAL REPORT

PROJECT TITLE

Market Promotion for the Development of Cultured Marine Shrimp on Guam

GRANT PERIOD

July 1, 1992 through June 1994

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INTRODUCTION

Aquaculture production and marketing development have often confronted the same chronological dilemna as the proverbial chicken and the egg question; namely, which comes first. If a market exists but the desired product cannot be produced cost effectively, a business cannot produce profit. Similarly, it is commonly assumed that "marketing" will occur spontaneously as the products "sell themselves". As has been discovered by all too many aquaculture firms entering the marketplace with a new product, a product with no market can result in poor cash flow and business failure. Production and marketing must go hand in hand, or neither can fully succeed.

The aquaculture industry on Guam has experienced this same fundamental development dilemna because production and marketing have not been coordinated. On Guam, as has usually been the case elsewhere, development efforts have focused on production, while marketing has been, at best, an afterthought. Farmers on Guam have been successful at producing surpluses of tilapia, catfish, and milkfish, with little or no planned concurrent market development.

Where market research has been conducted, it focused primarily on the scholarly task of gathering information on the market potential of hypothetical culture species or product forms. While this is a good first step and forms the basis for considering future production, it does little to develop the actual linkage between the producer and the final consumer. Without the development of market linkages, even the best of aquaculture products may never leave the farm, and efforts to expand production may be for naught.

The marketing project described here was created as a component of the overall Guam Aquaculture Development and Training Center (GADTC) goal to foster aquaculture through the coordinated approach of seedstock production, research (production and marketing), and technical assistance. In this case, the three-pronged GADTC approach was employed to introduce the marine shrimp, *Penaeus stylirostris*, to farmers and markets on Guam. Specifically, this project focused on the components of refining market sector identification and establishing critical market linkages between producers and consumers.

The culture of *P. stylirostris* on Guam was initiated in 1990 following the identification of candidate species with high production and market potential under local conditions. Shipments of mature blue shrimp were brought to Guam from Hawaii and spawned at the GADTC hatchery to produce populations that would grow to become the hatchery broodstock. By June 1991, the first seedstock from the hatchery was stocked into local farm ponds for growout demonstrations.

The highly successful farm trials, coupled with preliminary studies of established market channels, indicated that *P. stylirostris* culture could succeed on Guam as expected. By early 1992, farmers were producing enough shrimp to approach market saturation through traditional retail outlets, and realized that further market development would allow expanded production.

Of several potential alternative markets for aquaculture products, the substantial tourist industry on Guam was identified as the most promising target for immediate and long-term market expansion. Although a number of potential markets to access tourists exist, the most natural market avenue for bringing aquaculture products to the tourists was through the established tourist oriented restaurants. Aquaculture farmers on Guam had previously supplied products to restaurants only on a sporadic and volumetrically insignificant basis. The restaurants purchasing aquaculture products typically served Chinese and Filipino cuisine to local clientele. Aquaculture products had never before been sold on a regular basis to the mainstream tourist oriented restaurants.

There are several reasons why this seemingly obvious market for aquaculture products has remained untapped on Guam. The primary species cultured in Guam until the 1990's; tilapia, milkfish, and catfish, were sold directly to consumers at convenient farmer operated retail outlets as whole live or chilled fish. No

processing, packaging, deliveries, or complicated billing procedures were needed, resulting in a very simple marketing function for the farmer. As is frequently the case elsewhere, the existing farmers on Guam are production oriented and prefer to keep marketing as simple as possible.

In addition, the price received by the farmer when selling directly to the consumer was often substantially higher than could be expected when selling to a middleman, such as a restaurant or retail store. The customer base on Guam was willing to pay a premium for live or very fresh fish from a local farm.

The direct marketing of whole, fresh fish to consumers was stimulated by demand that outstripped supply, at least until the late 1980's, so farmers had little incentive to open new markets. By 1988, local tilapia production had risen to a point near market saturation, causing intense inter-farm competition and price depression. Also at about that time, high quality frozen milkfish from Taiwan began to be imported into Guam, resulting in significant product substitution and lower demand for locally produced milkfish. It was at this time that the need for market expansion became apparent to the farmers.

The above mentioned species also had limited appeal in the tourist restaurants. Most tourists and local restaurant customers alike were primarily interested in ocean entrees such as tuna, mahi mahi, grouper, or lobster.

Finally, most restaurant chefs wanted a convenient, consistent product to serve their clientele. With the exception of the Chinese and Filipino chefs mentioned above, they were as unwilling to process whole fish as the farmers were, for the same cost-conscience reasons. Restaurant buyers were also reluctant to buy fresh product from a local producer when a pre-processed, consistently sized frozen product was reliably available from wholesalers.

Cultured marine shrimp provided solutions to many of the constraints that hindered the entrance of local aquaculture products into the restaurant market. Most importantly, shrimp were very appealing to customers and chefs as a whole, fresh product, therefore the added product handling and costs were unnecessary. In addition, the quality of fresh, locally raised shrimp could not be matched by imported products, for which there was already a substantial demand.

With locally raised marine shrimp in a favorable position to enter the restaurant market, this project was implemented to form the critical market linkages between the producers and the previously untapped restaurant market sector.

PROJECT OBJECTIVES

The project addresses specific objectives under market promotions in the development of marine shrimp culture as follows:

- To identify specific demands and requirements of the tourist market sector for marine shrimp,
- To categorize components within the tourism market to identify niches by product form demand,
- To develop a marketing strategy to maximize market penetration of the tourism market sector on Guam,

- To develop appropriate promotional literature for restaurants,
- To develop an exhibit for trade shows,
- To coordinate the demands for the product by the potential market categories with the production capability of the commercial farms.

METHODS

The overall project was compartmentalized into three integrated sub-projects to allow flexibility in responding to the development of the previously untapped restaurant market. The three sub-projects overlap to some degree in satisfying the objectives of the proposal. It is convenient to view the overall project in components in order to focus on specific accomplishments. In addition, each sub-project generated separate reports that have been compiled in the appendices of this final Project Report.

The three sub-projects conducted focused on the following project aspects:

1. Market Sector Identification.
2. Product Promotion.
3. Market Linkage Facilitation.

RESULTS

Market Sector Identification

Identification of the product form and demand for fresh, locally cultured marine shrimp in the tourist sector on Guam was the first sub-project conducted under the umbrella of the overall project. This preliminary effort was commenced in March 1992 at the onset of the project with the goal of gathering specific information on the demand that existed in the restaurant marketplace for locally cultured shrimp. A group of sixteen prominent restaurants was selected as a representative pool from which to gather the shrimp marketing information. Samples of the fresh cultured product were delivered to each of the restaurants, and information was gathered from each regarding the quality, size, price, form, delivery, and purchasing protocol preferred for the shrimp. The specific methods and results of the project are reported in Appendix I, Preliminary Introduction of Locally Raised Shrimp to Hotels and Restaurants on Guam.

The results from this initial sub-project provided valuable insights into the complexities of the untapped restaurant market for shrimp (and other aquaculture products). Most encouraging was the unanimous agreement among restauranteers on the superior quality of the locally cultured product. The preferred sizes, product forms, and acceptable price ranges for each were identified. In addition, information was gathered to form the basis of segmenting the overall restaurant market into specific sectors based on business characteristics and clientele served. This identification of markets niches provided the framework for formulation of marketing strategies for reaching the tourist market.

The results from this project also provided aquaculture farmers with a rationale for thoughtful expansion of shrimp production. This information provided guidelines for market driven production into the new restaurant sector, substantially lowering the risk of entering new market territory.

Product Promotion

Promotion of the new aquaculture product of marine shrimp to restaurants and the general public was the thrust of the second sub-project nested within this overall project. The uninitiated may at first perceive that the promotion of cultured local shrimp would be somewhat superfluous, owing to the expected quality and nearly universal appeal of fresh shrimp. This perception is an outgrowth of the frequently encountered belief that good products will "sell themselves", which is a marketing fallacy that can be fatal to even the best products.

Although the excellent quality of the shrimp was universally recognized by the targeted restaurant sector, product promotion goes far beyond establishing quality recognition. As was suspected prior to the project and reinforced in the initial sub-project, virtually nothing was known of the local aquaculture industry in the previously untapped restaurant marketplace. Nor was there much knowledge of local aquaculture in other market sectors on Guam, with the exception of the well defined fresh, whole fish market sector dominated by local Filipinos and Chinese.

The promotion of the fresh marine shrimp was, therefore, designed to educate the target audience of restaurant buyers, tourists, and the general public about this exciting new product on Guam. Two keystone events and an ongoing promotional program were used in this project to promote fresh cultured shrimp to the target audiences.

The first event, a special tasting of locally cultured shrimp at a prominent gourmet restaurant, occurred in October 1992. The event was hosted and advertised by the President of the Micronesia Chefs Association, and attended by a cross-section of chefs and food buyers from the restaurant industry. Several preparations of the shrimp were served, and a slide presentation and talk were given to inform the audience about shrimp culture on Guam. Promotional literature on shrimp aquaculture and the final product was distributed. A full report on the event is provided in Appendix II, Hotel and Restaurant Marketing Project: Blue Shrimp Tasting.

The second event in April 1993 is summarized in Appendix III, Fresh Product Showcase Report. In this event, fresh cultured shrimp were again featured in a culinary display where restauranteers could sample a variety of premium products. Promotional "table tents" designed for the project to promote shrimp were introduced at this meeting. These table tents were subsequently used by several of the restaurants that began to purchase the fresh shrimp. They alerted restaurant clientele to the availability of the new, fresh, local product, and promoted local aquaculture in general.

The ongoing promotional program of the project involved a two-pronged approach. Media attention was drawn to locally cultured shrimp through regular press releases about special events and shrimp culture at the GADTC hatchery and the local farms. Several newspaper, magazine, radio, and television spots were generated that help to educate the general public about the availability of the new cultured shrimp.

A second aspect of the ongoing promotional program was the development of a

display that could be used for shrimp promotion and aquaculture education. Materials and text for the display were collected and refined over the course of the project. The display is portable and flexible for easy transport and convenient interchange or refinement of information. The display is designed for trade shows, conferences, meetings, and other similar mass communication venues, where sharing information about shrimp culture and products is appropriate.

Market Linkage Faciliation

Facilitation of the establishment of critical market linkages between the shrimp producers and the restaurant marketplace was the third sub-project of the overall project. This aspect of the marketing process was the most important component of project success. Although the restaurants were aware of the product availability and quality following the promotional components of the project, they were still hesitant to purchase shrimp from local producers. This reluctance can be summarized as a lack of confidence in an unfamiliar market linkage.

The lack of confidence is explained by actual negative experiences and an understandable aversion to risk. Food buyers in major restaurants on Guam have essentially no experience in buying aquaculture products from local producers (since this market sector has been virtually ignored until now). However, they have all had experience buying fruit and vegetables from local farmers, and have found the supply to be predictably unreliable in both quality and availability. This unreliability stems from a variety of reasons. Unfortunately, local aquaculture farmers are collectively perceived as having the same inability to supply the restaurants.

Restaurants on Guam historically have purchased shrimp as a frozen product from wholesalers. The product is conveniently purchased, easy to handle, in the desired form, properly sized, reliably available, and easy to store. The quality advantage of a fresh product does not, by itself, outweigh the combined assets of the frozen product. Most food buyers prefer to avoid the risk of switching to a new, unfamiliar supplier, and stay with a product they can depend on.

On the other side of the market equation, the producers are reluctant to incur the additional costs and management effort required to deliver a product to the restaurants according to their precise needs. This separation between restaurant buyers and shrimp producers created a challenging environment for the establishment of market linkages.

In recognition of this gap, bringing the buyers and producers closer together was an underlying theme of all project activities. In the Market Sector Identification sub-project, information provided to the restaurant buyers was tailored to inform them about the aquaculture farmer's ability to produce a consistent, reliable, quality fresh shrimp.

During meetings of the Product Promotion sub-project, Buyers were brought together with producers for discussions of concerns, to build communication bridges and to establish familiarity between the two market components. Chefs were invited to visit farms and the GADTC hatchery so they understood production. Meetings were facilitated between farmers and restaurant staff to discuss business opportunities.

The culmination of the effort to establish market linkages was the completion of a sub-project component summarized in Appendix IV, Promotion of Locally Cultured Blue Shrimp in Restaurants on Guam. In this portion, three restaurants were selected from the High Profile Sector (see report) for participation in a guided shrimp promotion. Each of the restaurants were supplied with a limited quantity of shrimp, and required to run mass media advertising to attract customers and attention to the cultured shrimp. Restaurants were supplied with tabletop advertising tents, and feedback on the success of the promotions was closely monitored. The result of this promotion was that all three restaurants made commitments to buy the fresh shrimp on a continuing basis.

As project events were being implemented, a steady stream of information about the new market was being generated that was directed toward shrimp producers to educate them about the requirements of the new market, and to help guide their shrimp production decisions. This vital linkage was facilitated by the GADTC project staff during the early stages of the project. As the project progressed, the direct linkage between the restaurants and the producers strengthened and by the end of the project GADTC facilitation became less necessary.

A positive spin-off of the project was the identification, in both marketing and production areas, of the need for further research that would support aquaculture development. The dialog created between shrimp producers and restaurant buyers identified several areas of uncertainty where additional research would be helpful.

SUMMARY

In summary, the three sub-projects of Market Sector Identification, Product Promotion, and Market Linkage Facilitation succeeded in moving the new aquaculture product of marine shrimp from the farm gate into an entirely new market on Guam. This was accomplished by directing a coordinated series of efforts at the targeted market sector (tourists via restaurants), and producing a stream of feedback information that was valuable to the shrimp producers. The role played by the GADTC project staff in facilitating this two-way process was essential in the entry of an aquaculture product (marine shrimp) into an entirely new market (high profile restaurants) on Guam.

This project serves as a general model for the introduction of new aquaculture products into new markets on Guam. The critical element of the project success was the coordination of production and market development through facilitation of producer-buyer market linkages.

As a postscript, the project was completed in spite of severe typhoon damage to shrimp production during the latter half of 1992, and the funding delays experienced during the proposed time frame of the project (see quarterly reports).

APPENDIX I

PRELIMINARY INTRODUCTION OF LOCALLY RAISED SHRIMP TO HOTELS AND RESTAURANTS ON GUAM

Preliminary Introduction of Locally Raised Shrimp to Hotels and Restaurants on Guam

By: David J. Landkamer

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OF THE

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Preliminary Introduction of Locally Raised Shrimp to Hotels and Restaurants on Guam

by David J. Landkamer
April 1992

Introduction

Aquaculture producers on Guam currently sell nearly all of their products directly to consumers at their retail stores or at the Harmon Flea Market. A majority of the customers are of Philippino descent. While this marketing strategy has been generally quite successful, the clientele base is limited and substantial competition between aquaculture producers exists. Recently, high quality frozen milkfish imported from Taiwan has also intensified competition and damaged prices and volume of local aquaculture products.

There are several alternative markets for aquaculture products on Guam such as retail grocery stores and specialty fish markets, the military outlets, institutions like the public schools, and hotels and restaurants. Developing one or more of these markets would stimulate increased local aquaculture production by expanding the customer base and reducing competition between producers.

A second factor constraining market development for aquaculture products is the limited appeal of currently produced species. The Guam Aquaculture Development and Training Center (GADTC) is responding to this constraint by producing seedstock of new species and by providing technical assistance to grow and market them.

Seedstock of the blue shrimp, Penaeus stylirostris, were produced at the GADTC, and, in 1991, a successful growout project was conducted at a private aquaculture farm to demonstrate production methods. Estimates indicate that over 100,000 pounds of shrimp could be produced annually at the farm where the demonstration was conducted. With production capabilities identified, the market potential needed clarification. Shrimp were first test marketed in the traditional flea market and retail market outlets, where they were very well received.

Because of a strong market demand, blue shrimp are one of the species that appears to have substantial potential for local aquaculture market expansion and diversification on Guam. During the first six months of 1988, $ 1.23 million of shrimp were imported into Guam. If this number is representative of shrimp imports, approximately 250 metric tons of shrimp are imported into Guam annually. Shrimp are purchased by a broad cross section of consumers through a variety of market channels. Fresh, high quality, locally produced shrimp could be readily substituted for imported shrimp in

some of the market niches. The hotel and restaurant market, having an acute appreciation for quality, seemed like a natural sector to develop for the sale of locally raised shrimp.

Objectives

The overall purpose of the project was to assess the feasibility of entering the hotel and restaurant market sector with a locally grown aquaculture product. The specific objectives were:

1) To introduce the food and beverage management at hotels and restaurants on Guam to local farm-raised blue shrimp,

2) To determine the importance of shrimp quality, size, and product form to restaurant buyers on Guam,

3) To assess the price range that restauranteers are willing to pay for fresh shrimp,

4) To determine the volume of shrimp used by local hotels and restaurants,

5) To explore other factors that influence hotel and restaurant purchasing,

6) To raise awareness among Guam food service management of locally produced aquaculture products,

7) To increase hotel and restaurant confidence in the reliability and consistency of locally grown aquaculture products.

Methods

The introduction of locally grown shrimp to hotels and restaurants began with the identification of established businesses where shrimp were being served. From this group, 16 candidate restaurants were selected for participation in the shrimp introduction project (Table 1). Most of the establishments selected were major hotels or "white tablecloth" restaurants that seemed most likely to appreciate the high quality, locally raised shrimp.

Table I

Hotels and Restaurants Particpating in the Guam-Raised Shrimp Introduction Project

Fujita Guam Tumon Beach Hotel	Korea Palace
Guam Palace Hotel	Ladera Inn
Guam Hilton Hotel	Pacific Islands Club
Guam Okura Hotel	Pacific Star Hotel
Hotel Nikko Guam	Sandcastle
Istemiwa Restaurant	Seahorse
Japan Plaza Hotel	Sotetsu Tropicana Hotel
Joinus Restaurant	Tinamarie's

Selected restaurants were initially contacted by telephone and the individual responsible for purchasing was identified. Depending on the business, this was most often either the purchasing manager, the food and beverage director, or the executive chef. Following introductions, the role of the Department of Commerce and the Guam Aquaculture Development and Training Center in supporting the growth of local aquaculture was explained. The restaurant's interest in fresh shrimp was confirmed, and an appointment was arranged for delivery of a sample of the blue shrimp to each. Some of the hotels requested multiple samples to distribute among their many chefs and restaurants.

From March 12 to 16, 1992, samples of shrimp were delivered to each of the 16 restaurants. Shrimp were harvested from GADTC hatchery pond S-7 using dip nets, and chilled immediately in ice water. Chilled shrimp were drip dried for three minutes in a large colander, weighed, counted, and placed in styrofoam "six-pack" coolers on ice. For individual restaurants, each cooler contained about four pounds (approximately 100 shrimp) which averaged 18.6 grams (24.4/lb). For hotels requesting several samples, a sufficient number of one gallon plastic ziplock bags were filled with about 2 pounds of chilled shrimp each and placed in a cooler on top of about 2 pounds of shrimp on ice. Each cooler was made to look attractive and full, using ice under and on top of the shrimp.

Samples were delivered to the arranged contact person at each establishment. Initial buyer reactions to shrimp color, size, and quality were noted. Contacts were asked what size shrimp they currently use, what price range they pay, and what quantity they purchase each week or month (Appendix 1). Often the fresh shrimp were eaten raw, or cooked then eaten during the first meeting to check product quality.

Supporting written materials were delivered with the shrimp to each of the restaurants (Appendix 2). These included a letter introducing blue shrimp and explaining the GADTC role in aquaculture, a blue shrimp product fact sheet, a GADTC hatchery glossy, and a GADTC business card.

Each establishment was called on the telephone 2 to 8 days after the sample delivery to gather additional information about the restaurant reactions to fresh blue shrimp. Questions about quality, size, price, form, delivery, and purchasing contracts were asked of each restaurant representative (Appendix 3).

Finally, about three weeks following the sample delivery, a letter was sent to each of the dining establishments to thank them for their reactions to the blue shrimp, to remind them that they will be contacted again in June to negotiate shrimp purchases, and to welcome them to visit the GADTC by calling to arrange a tour.

Results and Conclusions

The reaction to the fresh blue shrimp was very positive overall. Practically all of the buyers expressed dismay over the extremely limited selection of seafood and produce that could be purchased from local fishermen and aquaculture producers. In spite of being an island with seemingly substantial fishery resources, the only local seafoods available to the restaurants on a regular basis are tuna and mahi mahi. For this reason, the availability of a locally produced, premium quality fresh shrimp was very exciting to them.

In every case, the quality of the blue shrimp was judged to be superior to any other shrimp the restaurants have access to on Guam. Quality descriptors for the taste of the shrimp offered by the buyers included good, very good, excellent, outstanding, sweet, delicious, and " better than any other shrimp available". The color was also viewed very favorably, both before and after cooking. Clear, translucent color of the fresh shrimp, and light salmon-pink color of the cooked shrimp were viewed as very favorable. The very high quality of the product was not disputed by any of the buyers.

The possible use of blue shrimp for sushi was evaluated by several of the chefs at hotel restaurants. The availability of a very fresh or live product was of great interest. For most, the freshness, color, texture, and taste were acceptable. One of the chefs felt that the blue shrimp was very good, but not as sweet as the North Atlantic frozen shrimp currently used.

The species of shrimp used by the restaurants did not seem to be an important factor. While most buyers and chefs were aware of some of the different species commonly offered, they did not usually make a distinction between them as to taste or color preferences (with the exception of the above example). Only marine shrimp, however,

and not freshwater prawns, were used by any of the restaurants contacted. The black tiger prawn, Penaeus monodon, was by far the most commonly used species, followed by pink shrimp and occasionally, flower, green tiger, banana, and white shrimp. Nearly all of the shrimp used by restaurants originated from farms in the Philippines.

The conventions used in the industry for sizing and packaging of shrimp are fairly complicated. In fact, many of the restaurant buyers did not understand the meaning of the size designation numbers for shrimp on the wholesaler's price lists or on the packing boxes. The size ranges used commonly by the restaurant trade to grade whole shrimp and the corresponding weights of individual shrimp in each size range are provided in Table 2.

Two methods for designating shrimp size are commonly used. Shrimp are most often packed in boxes of 1 to 4.4 pounds (.45 to 2 kg) according to established number of shrimp per pound categories. For example, the 16/20 designation means that there are 16 to 20 shrimp per pound in the package. All of the shrimp are approximately the same size, with some variation within the range.

Alternatively, shrimp of very uniform size are often packed according to the number of pieces per box. For example, a "15 count" shrimp in a 2.87 pound box indicates that there are 15 equal sized shrimp per 2.87 lb box (86.9 grams/shrimp, 5.2 shrimp/lb). The 2.87 pound (1.3 kg) weight is a common packing standard, although the origin of its use is unclear.

Shrimp sizing becomes more confusing when other product forms are considered. For example, when 8/12, 13/15, etc., designations are used for headless shrimp, the numbers indicate the size of the tails rather than the size of the whole shrimp before removal of the head. Therefore, an 8/12 headless shrimp is a much larger tail than an 8/12 head-on shrimp.

The reactions to the size of the shrimp (25/lb) included all possible positive and negative responses from too small to perfect to too large. In general, the buyers would prefer a larger shrimp of up to 5/lb. Some of the restaurants currently use the 21 - 25/lb or the 26 - 30/lb size and were very pleased with the size of the shrimp in the sample. A slightly larger product in the 16 - 20/lb range appeared to be more acceptable in most cases. Others would use a whole shrimp only in a smaller size, usually 31 - 40/lb, 41 - 50/lb, or 51 - 60/lb.

Most of the restaurants were looking for a shrimp sized to fit their existing uses, while a few expressed an interest in modifying their menus to use the fresh shrimp at the sample size offered. It is clear that one or even three to four sizes will not be adequate to address all the restaurant market niches. In fact, several sizes will probably be needed to satisfy all of the uses of shrimp at any one of the restaurants.

Table 2

Commonly Used Shrimp Size Grades

Size Designation Grade	Weight (grams) of Shrimp Within	
	Range	Average
8/12 per lb	36.3 - 60.5	45.4
13/15 per lb	29.3 - 36.2	32.4
16/20 per lb	22.2 - 29.2	25.2
21/25 per lb	17.8 - 22.1	19.7
26/30 per lb	14.9 - 17.7	16.2
31/40 per lb	11.2 - 14.8	12.8
41/50 per lb	9.0 - 11.1	10.1
51/60 per lb	7.5 - 8.9	8.7
61/70 per lb	6.5 - 7.4	6.9
71/90 per lb	5.0 - 6.4	5.7
100/200 per lb	2.3 - 4.5	3.4
12 count	see note	108.7
15 count	"	86.7
18 count	"	72.2
20 count	"	65.0
25 count	"	52.0
30 count	"	43.5
35 count	"	37.3
40 count	"	32.7

Note: Average weights were not measured, however, chefs often preferred the "count" shrimp because sizing was much more consistent than withthe "per pound" sizing.

A frequently repeated concern of all buyers was that the shrimp, regardless of size, be consistently sized within an order and between orders. In most cases, restaurants strive to present their customers with the same plate of food in taste and appearance every time it is ordered. Very low size variation in shrimp populations or size sorting prior to delivery will be necessary in many situations to satisfy the consistency requirements of the restaurants.

Product form preferences were also extremely varied. All of the restaurants contacted currently use a frozen product purchased (in nearly every case) from one local wholesaler. Most would prefer a fresh product, although a few indicated that they preferred the convenience of a frozen shrimp, which provided a more consistent product to meet the unpredictable restaurant demand. None of the restaurants used the imported chilled shrimp found in some of the local seafood retail markets. While the delivery of fresh shrimp should remain the focus of local producers, providing a high quality frozen product would alleviate the concern that restaurants have of always maintaining enough inventory on hand of the same shrimp to serve unpredictable customer demands, while not over-buying the fresh, persihable product.

Shrimp are available in numerous processed forms for restaurant use, with each form having a common designation in the industry. The most common designations used to identify shrimp product forms used on Guam are listed in Table 3.

Table 3.

Designations Commonly Used for Shrimp Product Forms by the Seafood Industry on Guam.

Designation	Meaning
P&D	Headless, peeled, and deveined
Peeled	Headless, peeled, not deveined
PUD	Headless, peeled, not deveined
Peeled BKN	Headless, peeled, not deveined, and either some of the tails are broken into pieces, and/or the sizes are varied (broken size categories, not uniformsizes)
PND	Headless, peeled, not deveined
PND T/O	Headless, peeled, not deveined, with the telson and uropods ("tail") still on. This form is commonly used for tempura
HL	Headless, not peeled or deveined (letters preceding the HL designation refer to the variety of shrimp. For example, BTHL = black tiger headless, PHL = pink headless, etc.)
HO	Head on, or whole (preceding letters apply as above)
IQF	Individually quick frozen

For larger shrimp (8 - 15/lb), the whole form was most common used. For smaller sizes, however, the headless or headless, peeled and deveined form was very frequently preferred. Several of the restaurants were unwilling to handle a whole product because of the added labor cost for heading and peeling the shrimp. There are well defined markets for processed shrimp in the forms mentioned above.

Two of the restaurants contacted expressed an unsolicited interest in live shrimp. One would consider displaying a small number of shrimp in an aquarium for diners to view. The second was interested in holding large quantities in a tank or aquarium for customers to select for culinary preparation. The cost for a farmer to deliver large volumes of live shrimp appears to be more than the restaurants would be willing to pay.

Price was very important to most of the buyers, while a few of them indicated that they would purchase the local, fresh product at any reasonable price. Prices currently paid by restaurants for frozen shrimp from wholesalers, as quoted by buyers, is presented in Table 4. The price list from the principal seafood wholesaler on island corroborates (approximately) the stated prices in every case.

The prices that restaurant buyers were willing to pay for fresh shrimp of the quality offered in the sample are listed in Table 5. In general, prices were based in nearly every case on the current price paid for a comparable frozen product from the local wholesaler. Buyers are willing to pay slightly more for the fresh product, but not substantially more.

The approximated price of $7.00/lb for 25/lb whole shrimp was initially met with either tolerance, reluctant acceptance, slight resistance, or complete rejection. Prices currently paid for a similar sized frozen product were reported to be between $5.25 and 5.50/lb by designated contacts. However, several of the restaurants were buying headless shrimp in this price range and expected to pay 25 to 33 % less for a whole shrimp. In spite of recognized quality, price (related to size and form) will be a major deciding factor in whether most of the restaurants buy locally raised shrimp.

Buyers were asked if a purchasing contract establishing minimum quantities and maximum prices would be of interest to them. All responded positively to the concept, indicating that frozen shrimp prices change every three weeks and sometimes fluctuate wildly. They were also concerned that the farmer's price might go up dramatically soon after they began buying, so a price contract was an attractive means to address that issue.

The quantity of shrimp used per week by each individual restaurant or hotel in the survey ranged from 0 to 500 pounds, which included all product sizes and forms. All together, the 16 restaurants used a reported 2,240 pounds of shrimp per week. Assuming these numbers to be relatively accurate, this volume translates to almost 53 metric tons/year.

Table 4

Shrimp Sizes and Forms Currently Used by Surveyed Restaurants

Product Form Paid	Sizes Used by Restaurants	Price/lb.
whole (HO)	13/15	$6.50
	16/20	6.00
	21/25	5.50
		5.25
	31/40	4.50
	15 ct, 2.87 lb/box	11.00
		10.25
	20 ct, 2.87 lb/box	8.25
	25 ct, 2.87 lb/box	7.88
	40 ct, 2.87 lb/box	5.00
headless (HL)	8/12	10.71
	13/15	9.65
	16/20	5.95
		7.45
		7.20
		7.40
		7.00
		6.95
	21/25	6.15
		5.75
	26/30	5.25
		5.23
		5.35
	31/40	4.90
	41/50	4.55
	51/60	4.25
headless, peeled and deveined (P&D)	8/12	8.25
	16/20	7.95
	26/30	6.85
	51/60	5.45
	100/200	2.75

Table 5

Stated Prices that Restaurant Buyers Would Pay for Fresh, Raised-on-Guam Shrimp

Product Form	Size	Prices Stated	Suggested Average
whole (HO)	15 ct	$11.00	$10.50
			10.00
	25 ct	8.00	8.00
	40 ct	5.00	5.00
	13/15	8.75	8.75
	16/20	7.00	6.69
			7.00
			6.50
			6.25
	21/25	7.00	6.63
			7.00
			6.50
			6.00
	26/30	6.50	6.17
			6.50
			5.50
	31/40	5.00	5.00
headless (HL)	8/12	8.00	8.00
	16/20	7.50	7.35
			7.20
	21/25	6.00	6.00
	26/30	6.00	6.00
			6.00
	31/40	5.50	5.50
headless, peeled,	6/8	8.50	8.50
deveined (P&D)	8/12	8.50	8.50
	26/30	7.00	7.00

If local production of shrimp were limited to initial recommended sizes and only the whole product form, it is estimated that about 20 to 25 % of the total hotel/restaurant market could be initially captured by the local product. This translates to an annual demand of nearly 30,000 pounds. Further customer expansion and increased sales per customer could more than double this demand. When surplus production to ensure continuous product flow is considered (which could be readily sold in the local flea market), Guam farmers should aim for a production level of between 75,000 and 100,000 pounds annually to successfully penetrate the hotel/restaurant market sector.

When asked how frequently the product should be delivered to meet their needs, most buyers indicated that daily deliveries would be best, but that 2 to 3 deliveries/week would be adequate. Of the 16 hotels/restaurants surveyed, 3 preferred daily deliveries, 4 preferred 3 deliveries/week, 7 preferred 2 deliveries per week, and 2 indicated that 1 delivery/week would suffice.

Many buyers were pleased that shrimp could be produced locally and expressed a keen interest in supporting local producers. Locally raised, fresh shrimp is a strong selling point that is worth promoting. A major concern, however, was whether the local producers could provide a consistent, reliable supply of product on a continuous basis, and at a stable price. Many of the restaurants have had bad experiences with local producers of agriculture crops in the past. Providing assurances of product continuity and delivering product as promised will be critical to accessing and maintaining restaurant market niches.

Recommendations

Based on the preliminary information gathered in this shrimp marketing project, the following is a list of recommendations that should be considered by local farmers intending to penetrate the hotel/restaurant market with a locally raised shrimp product.

- Producers must take all necessary steps during harvest, post-harvest, and delivery to insure the freshness and quality of locally produced shrimp reaching the restaurants. This includes harvesting the shrimp as soon as possible prior to delivery, handling the shrimp carefully to minimize breakage and damage, chilling the shrimp immediately after harvest, and transporting the shrimp on ice. High quality and freshness are the principal features of the locally grown product that distinguish it from competing imports.
- The recommended product form for farmers to produce initially is the whole, fresh shrimp. This approach is the simplest and has numerous merits. Fresh, whole shrimp will have a limited amount of direct product competition in the marketplace. Producing whole shrimp will require less processing labor and keep capital costs for processing equipment (heading machines, graders, freezers, etc.) to a minimum. Limiting product offerings will also minimize management and labor costs. Problems with product continuity are less likely with fewer product forms.

- Frozen whole shrimp should be considered as a product form if restaurants have a difficult time maintaining fresh shrimp inventories due to unpredictable fluctuations in customer numbers and demand for shrimp. Alternatively, restaurants could be encouraged to freeze a "reserve" of the fresh shrimp themselves for use in the event of an unanticipated customer demand for the product.

- Processing (heading or peeling) is not recommended until the market demand for whole shrimp is completely satisfied and there is a substantial surplus of shrimp produced in order for the farmer to benefit from the economies of scale. Oddly, the headless shrimp sell for up to 20% more per pound than the same sized whole shrimp, but the entire shrimp weighs between 30 to 40% more than the tail alone. Processing actually reduces the return per pound, although a processed product would certainly reach markets beyond those accessible to whole shrimp. (The lower price for processed shrimp is due primarily to the use of lower quality shrimp for processing. If shrimp arrive at the processor in excellent condition, they are most often packed as whole shrimp. Lesser quality shrimp are salvaged by processing.)

- There is a demand for many different sizes of shrimp in the marketplace. Rather than attempt to fill all the size niches right away, producers should at first offer two or three popular sizes until reliable production is well established. The small to medium sizes are suggested because shrimp will reach marketable size sooner, thus providing a shorter growout time and a more rapid generation of cash flow for the farmer. The first size ranges produced should be the 26 - 30/lb, 21 - 25/lb, and 16 - 20/lb. Of course, smaller sizes will be available if the farmer wishes to expand size offerings. Larger sizes can follow as production increases, and depending on the farmer's willingness to diversify.

While many of the restaurants would prefer shrimp packed according to the "count" method (ie.; 15 count, 20 count, etc.) because size is more uniform, the extra labor, management, and packaging costs for the farmer would be substantial. The count method may be necessary for the largest sizes, but for the starting sizes recommended, the number/lb convention is much simpler and will suffice.

- Shrimp should be sized extremely carefully and consistently, both within a delivered lot and between lots. While individual blue shrimp in a given pond typically grow at a more consistant rate than some other species, the size variation within a population may be too great for restaurants to accept.

Even the size range found within conventional shrimp size grades (ie., 16 -20/lb, 21 - 25/lb, etc.) may be unacceptable to many restaurant if encountered within a single lot. For example, shrimp in the 16 - 20/lb size would range from 22.2 - 29.3 grams each. Most restaurants appear to be tolerant of a 2 - 4 gram weight spread, but not the 7 plus gram spread found in this size grade.

Farmers should develop convenient tools for judging individual shrimp size to ensure consistency. Initially, shrimp should be size graded by hand to remove animals that are not within the acceptable size range. As production volume expands, farmers may want to consider mechanical grading of shrimp, although it may prove too harsh for the preservation of product quality and is not recommended in the beginning.

Alternatively, producers could offer "pond run" grade shrimp that have not been sized, at a lower price. This would limit the market for the product to some extent, but greatly simplify handling and reduce labor costs for the farmer.

- The price for locally grown shrimp should be higher than that for comparable frozen imports to reflect the excellent quality and freshness, but not so high as to discourage local buyers. Based only on input from restaurants as to what they said they would pay for locally produced shrimp, price ranges for some sizes of whole shrimp are recommended (Table 6). (Production costs are not taken into consideration in these pricing suggestions.)

Table 6

Suggested Price Ranges for Some Sizes of Locally Produced, Whole Shrimp Being Sold to Restaurants.

Size/Grade of Whole Shrimp	Average Price/lb Restaurant Said They Would Pay	Suggested Wholesale Price Range/lb for Locally Raised Shrimp
13/15	$8.75	$8.00 - 9.00
16/20	6.69	6.75 - 7.50
21/25	6.63	6.50 - 7.00
26/30	6.17	6.00 - 6.50
31/40	5.00	5.50 - 6.00

- A price structure for the shrimp, dependent on size, volume, and other factors, should be established that can be held constant for several months at a time. Price stability is very attractive to restaurant buyers, particularly due to wide fluctuations in the price of imported frozen shrimp.

- Market expansion into the restaurant sector should occur in a deliberate and controlled fashion, based on a steady and reliable production of product. Market expansion beyond production capacity will undermine the efforts of local aquaculture farmers to establish firm access to restaurant markets and discourage future import product substitution.

Further Shrimp Marketing Needs

This preliminary introduction of blue shrimp to the hotel and restaurant market on Guam indicates a need for a wide diversity of research and promotional activities to more accurately identify and penetrate local markets with aquaculture products. The most obvious needs are direct outgrowths of and follow-ups to this project including:

» Development of appropriate harvesting,chilling, and tranporting methods for whole shrimp;

» Refinement of shrimp grading methods;

» Production cost research to identify break even prices;

» Identification of greatest return price structures for variuos sizes of shrimp;

» Implementation of product identification and promotion project;

» Freezing technology research;

» Heading technology research;

» Refinement of methods for transporting and holding live restaurant-size shrimp;

» Benefits of labeling aquaculture products with the Guam Product Seal;

» Development of packaging systems to maintain product quality, identify product, and minimize packaging costs;

» A number of additional potential project topics emerged from this study including;

» Identification and research of alternative fresh shrimp markets;

» Introduction of additional aquaculture species into the hotel restaurant market;

- Optimum pond stocking density for blue shrimp on Guam;
- Low-cost shrimp predator and competitor eradication methods;
- Research to identify shrimp product forms and quantities imported into Guam.

- Price competition from imported frozen shrimp is likely to be strong. There is currently one seafood wholesaler that supplies the bulk of the frozen imported shrimp currently supplied to restaurants on Guam. When the fresh, local product begins to capture a significant portion of the existing frozen market, prices for frozen product are expected to drop substantially. Producers should anticipate this price drop by promoting their local product vigorously, setting moderate prices initially, and establishing medium term (six month) price contracts with restaurants.

- There is a value premium attached to locally grown products, such as fish, shrimp, and produce. Producers should invest in the promotion of their shrimp as locally raised, grown on Guam aquaculture products. This will encourage the purchase of the shrimp by tourists and substantiate the price asked for the product.

- Fresh shrimp should be delivered 3 times/week to high volume customers so that product freshness can be assured. Although more frequent deliveries are preferred by some restaurants, deliveries should be limited to minimize costs as much as possible. More frequent deliveries may be appropriate as volume increases.

A packaging system should be developed to ensure a fresh, high quality product delivered in a restaurant "friendly" format. The packaging should be well integrated with farm harvesting methods, be cost efficient, and be marked to clearly reinforce the local origin of the product.

- Maintaining a steady stream of product supply is absolutely critical. If a farmer misses just one delivery due to lack of supply (or for any other perceivably avoidable reason), the restaurant buyer will likely revert to a more reliable supply of frozen product. Producers should initially focus on providing shrimp to a limited number of restaurant outlets. As production expands, additional restaurants should be contacted to identify avenues for market expansion.

Production should be planned accordingly to generate a steady flow of appropriately sized shrimp to exceed the restaurant demand. Farmers should anticipate an annual production of near 50,000 pounds in the first year to be able to satisfy the already identified hotel/restaurant demand for whole shrimp in the small to medium size range. Adequate production must be maintained to prevent interruptions in product flow. Surplus shrimp can be sold in alternative outlets, such as the flea markets or roadside stands, or be grown to a larger size for further market expansion.

APPENDIX II

HOTEL AND RESTAURANT PROJECT: BLUE SHRIMP

Hotel and Restaurant Marketing Project Blue Shrimp Tasting

TECHNICAL REPORT NO. 006

October 1992

TECHNICAL REPORT SERIES

OF THE

GUAM AQUACULTURE DEVELOPMENT AND TRAINING CENTER

DEPARTMENT OF COMMERCE
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Hotel and Restaurant Marketing Project
Blue Shrimp Tasting

7 October 1992

Introduction

In March and April 1992, a sample of blue shrimp was brought to each of several hotels and restaurants to determine their interest in this new, locally grown aquaculture product. The overwhelmingly positive response encouraged one local farm, Inarajan Aquaculture Enterprise (IAE), to gear up for full production of the shrimp. By October of 1992, IAE had several ponds stocked with shrimp of various ages and sizes, and was ready to begin harvesting upwards of 150 pounds per day.

Up until this time, shrimp, like other local aquaculture products, had been marketed through the traditional direct retail channels. The hotels and restaurants represented an entirely new market for local aquaculture products that would open the door for substantial production increases.

Although restaurant buyers were very interested in locally grown blue shrimp, there was still a need to match their needs with what the farmer would produce. To facilitate communication between IAE and the restaurant industry, and to initiate the sales of shrimp to restaurants, a special gathering was held. Bruce Grasley, President of the Micronesia Chef's Association, graciuosly offered to host the event at his Chianti restaurant, which was scheduled for the afternoon of Wednesday, October 7. All the restaurants that had been contacted in the spring, and several others that were contacted through the MCA, as well as the press, were invited.

Meeting Summary

On the morning of the meeting, shrimp were harvested from an IAE pond, chilled, and then sorted into three size categories as follows;

SIZE	GRAMS	APPROXIMATE NUMBER/POUND
S (Small)	11 - 15	31 - 40
M (Medium)	16 - 24	21 - 30
L (Large)	25 - 32	16 - 20

Seven pounds of each size, plus an additional 20 pounds of unsorted shrimp were delivered to the Chianti at 10:30 am to allow for preparation before the 3:00 meeting.

The tables at the Chianti were set as if for an elegant luncheon, with plates, silver, ice water and ice tea, bread sticks, and cloth napkins at each place setting. Each table also had a plate of each of the three sizes of shrimp, prepared by lightly boiling to accentuate the fresh flavor. The shrimp were served with a horseradish sauce and a tomato coctail sauce.

Hotel and restaurant guests arrived between 3:00 and 3:30, were greeted at the door by GADTC and IAE staff, and mixed freely while finding a table to sit at. At 3:45, Dave Landkamer welcomed the assembled group of 26, introduced the people involved in bringing the meeting about, and explained the afternoon's agenda. Represented hotels and restaurants included the Fujita, Pacific Star, Guam Palace, Leo Palace, Okura, Kamayan Pacific Islands Fisherman, Pacific Islands Club, and Hyatt.

Chef Bruce Grasley then talked about his impressions of the shrimp, and served two additional shrimp preparations using peeled tails; one in a garlic/butter sauce, and the other in an herbed tomato sauce. Everyone sampled the various preparations. His dishes were delicious and his commentary very positive.

Dave Landkamer continued with a slide presentation describing how blue shrimp are cultured on Guam, from hatchery production of PLs to farm growout and harvest. George Tsai then gave a brief overview of his aquaculture experience and sketched his ideas for supplying the restaurants with fresh shrimp.

A very informative discussion followed, led by Bruce Grasley, to help Mr. Tsai understand the needs of the restaurants. The chefs reiterated many of the same concerns that had been brought out in the spring sample deliveries, and summarized in the ensuing report. While very excited about the freshness and quality of the blue shrimp, most of the chefs were dismayed at IAE's reluctance to deliver the product as they wanted it. Specifically, the restaurants wanted meticulous size sorting, prompt delivery, and personalized service, while IAE's main concern was to minimize labor and marketing costs. Most chefs expressed a conditional interest in buying IAE shrimp, if IAE would take certain steps to meet their needs.

The meeting began to break up at about 4:30, with some of the chefs discussing specific issues about ordering the shrimp with Mr. Tsai. Chef Grasley provided a summary of insightful comments from his observations of the meeting, and encouraged Mr. Tsai to take some specific actions that will help him to enter the restaurant market.

Conclusions

The quality and desirability of fresh, grown-on Guam blue shrimp was resoundingly confirmed by the attending chefs, but they still had concerns about the service that would be provided with it. Several specific concerns that need to be addressed are as follows:

- in most cases, shrimp need to be sorted by size to be used by restaurants.
    - sizing must be very consistent within and between deliveries.
    - freshness is imperative. Chilled shrimp are top quality for a maximum of two days; therefore, deliveries will have to be a minimum of three times per week.
    - the supply must be consistently available in the future, without production gaps.
    - restaurants would like to able to identify their use of local shrimp on their menus or in their advertising to improve sales.
    - many of the restaurants would like small quantities delivered, such as 10 pounds.
    - each restaurant has a specific procedure for ordering and payment that must be followed. (Record keeping and billing will be somewhat complicated.)
    - price is a relatively minor concern. If all their needs are met, price is insignificant.

In summary, most of the restaurants like the blue shrimp, they want to use it, but they will take it only on their terms or not at all. The producer must be prepared to expend additional time and money to access this market, but can expect to be compensated for it. Local farmers who make the extra effort to satisfy customers will gain access to the new hotel and restaurant market.

Chef Bruce Grasley and the Micronesia Chef's Association were extremely supportive of our efforts to introduce blue shrimp to the restaurants on Guam. Efforts to include them in accessing the restaurant market are definitely worthwhile.

Several restaurants were not represented at the meeting, even though they had responded to confirm attendance. A follow-up event could be held to reach a broader audience. The media was also not present, mainly because, as it turned out, they did not receive their invitations until after the meeting (delivery took two weeks from when the letters were mailed!). Invitations should be hand delivered, as the restaurant invitations were, in the future. A special event just for the media would be worthwhile.

Hotel and Restaurant Marketing Project
Blue Shrimp Tasting

7 October 1992

Introduction

In March and April 1992, a sample of blue shrimp was brought to each of several hotels and restaurants to determine their interest in this new, locally grown aquaculture product. The overwhelmingly positive response encouraged one local farm, Inarajan Aquaculture Enterprise (IAE), to gear up for full production of the shrimp. By October of 1992, IAE had several ponds stocked with shrimp of various ages and sizes, and was ready to begin harvesting upwards of 150 pounds per day.

Up until this time, shrimp, like other local aquaculture products, had been marketed through the traditional direct retail channels. The hotels and restaurants represented an entirely new market for local aquaculture products that would open the door for substantial production increases.

Although restaurant buyers were very interested in locally grown blue shrimp, there was still a need to match their needs with what the farmer would produce. To facilitate communication between IAE and the restaurant industry, and to initiate the sales of shrimp to restaurants, a special gathering was held. Bruce Grasley, President of the Micronesia Chef's Association, graciuosly offered to host the event at his Chianti restaurant, which was scheduled for the afternoon of Wednesday, October 7. All the restaurants that had been contacted in the spring, and several others that were contacted through the MCA, as well as the press, were invited.

Meeting Summary

On the morning of the meeting, shrimp were harvested from an IAE pond, chilled, and then sorted into three size categories as follows;

SIZE	GRAMS	APPROXIMATE NUMBER/POUND
S (Small)	11 - 15	31 - 40
M (Medium)	16 - 24	21 - 30
L (Large)	25 - 32	16 - 20

Seven pounds of each size, plus an additional 20 pounds of unsorted shrimp were delivered to the Chianti at 10:30 am to allow for preparation before the 3:00 meeting.

The tables at the Chianti were set as if for an elegant luncheon, with plates, silver, ice water and ice tea, bread sticks, and cloth napkins at each place setting. Each table also had a plate of each of the three sizes of shrimp, prepared by lightly boiling to accentuate the fresh flavor. The shrimp were served with a horseradish sauce and a tomato coctail sauce.

Hotel and restaurant guests arrived between 3:00 and 3:30, were greeted at the door by GADTC and IAE staff, and mixed freely while finding a table to sit at. At 3:45, Dave Landkamer welcomed the assembled group of 26, introduced the people involved in bringing the meeting about, and explained the afternoon's agenda. Represented hotels and restaurants included the Fujita, Pacific Star, Guam Palace, Leo Palace, Okura, Kamayan Pacific Islands Fisherman, Pacific Islands Club, and Hyatt.

Chef Bruce Grasley then talked about his impressions of the shrimp, and served two additional shrimp preparations using peeled tails; one in a garlic/butter sauce, and the other in an herbed tomato sauce. Everyone sampled the various preparations. His dishes were delicious and his commentary very positive.

Dave Landkamer continued with a slide presentation describing how blue shrimp are cultured on Guam, from hatchery production of PLs to farm growout and harvest. George Tsai then gave a brief overview of his aquaculture experience and sketched his ideas for supplying the restaurants with fresh shrimp.

A very informative discussion followed, led by Bruce Grasley, to help Mr. Tsai understand the needs of the restaurants. The chefs reiterated many of the same concerns that had been brought out in the spring sample deliveries, and summarized in the ensuing report. While very excited about the freshness and quality of the blue shrimp, most of the chefs were dismayed at IAE's reluctance to deliver the product as they wanted it. Specifically, the restaurants wanted meticulous size sorting, prompt delivery, and personalized service, while IAE's main concern was to minimize labor and marketing costs. Most chefs expressed a conditional interest in buying IAE shrimp, if IAE would take certain steps to meet their needs.

The meeting began to break up at about 4:30, with some of the chefs discussing specific issues about ordering the shrimp with Mr. Tsai. Chef Grasley provided a summary of insightful comments from his observations of the meeting, and encouraged Mr. Tsai to take some specific actions that will help him to enter the restaurant market.

Conclusions

The quality and desirability of fresh, grown-on Guam blue shrimp was resoundingly confirmed by the attending chefs, but they still had concerns about the service that would be provided with it. Several specific concerns that need to be addressed are as follows:

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In summary, most of the restaurants like the blue shrimp, they want to use it, but they will take it only on their terms or not at all. The producer must be prepared to expend additional time and money to access this market, but can expect to be compensated for it. Local farmers who make the extra effort to satisfy customers will gain access to the new hotel and restaurant market.

Chef Bruce Grasley and the Micronesia Chef's Association were extremely supportive of our efforts to introduce blue shrimp to the restaurants on Guam. Efforts to include them in accessing the restaurant market are definitely worthwhile.

Several restaurants were not represented at the meeting, even though they had responded to confirm attendance. A follow-up event could be held to reach a broader audience. The media was also not present, mainly because, as it turned out, they did not receive their invitations until after the meeting (delivery took two weeks from when the letters were mailed!). Invitations should be hand delivered, as the restaurant invitations were, in the future. A special event just for the media would be worthwhile.

APPENDIX III

FRESH PRODUCT SHOWCASE PROJECT

Fresh Product Showcase Project

by: David J. Landkamer and Jerry Gardner

TECHNICAL REPORT NO. 007

April 1993

TECHNICAL REPORT SERIES

OF THE

GUAM AQUACULTURE DEVELOPMENT AND TRAINING CENTER

DEPARTMENT OF COMMERCE
Government of Guam
6th Floor ITC , Suite 601
590 South Marine Drive
Tamuning, GU 96911

FRESH PRODUCT SHOWCASE REPORT

Dave Landkamer and Jerry Gardner
26 April 1993

Introduction

On 26 April, 1993, an event called the Oyster Party was held at the Chianti Restaurant to showcase an assortment of fresh, high quality products available to chefs on Guam. The party has been an annual event for the past three years, organized and hosted by James Hutchison, President of Pan Pacific Fresh Foods Inc. on Guam. Pan Pacific is involved primarily with the import of fresh seafoods from Australia and New Zealand, but also is diversifying into fresh vegetables and spices.

James Hutchison invited the GADTC and George Tsai of Inarajan Aquaculture Enterprise to attend the event and represent locally farmed blue shrimp as a new product for Guam chefs. Pan Pacific has offered to wholesale the shrimp on Guam, including sorting and processing, if these market services would provide an opportunity for all parties to benefit.

Meeting Format

The Oyster Party was scheduled from 2:30 to 5:00 on Monday afternoon, the 26th. Plans were made by Mr. Hutchison to have the various representative products displayed buffet-style for participants to sample. Tables were set so participants could sit down to enjoy the samples and converse.

Bruce Grasley, executive chef at the Chianti, supervised the preparation and presentation of several seafood dishes from the showcased products. Attendees were to learn about the products by tasting and mixing with the vendors. No formal presentations were planned.

Pan Pacific brought oysters, littleneck clams, calamari, and mussels, which were prepared in an assortment of delicious ways. The GADTC delivered 25 pounds of shrimp (22/lb size) early in the day, which were also served in several tasty preparations. An additional 5 pounds delivered at 2:30 was boiled and lightly seasoned to augment the ultra-fresh taste.

Jungle Java fresh roasted coffee was ground, perked, and served to order. An assortment of wines from Chile and Spain were available for sampling.

The GADTC staff brought blue shrimp table tents promoting the local, fresh product and placed them at each of the tables, and near the shrimp dishes in the buffet where possible. A giant table tent (11 1/2 x 17") was displayed at the table where GADTC staff were seated, along with printed materials describing the shrimp and the GADTC hatchery for attendees to take.

In addition, GADTC staff circulated among the participants for the purpose of drawing attention to the farmed shrimp, and to introduce local farmers to chefs.

Inarajan Aquaculture Enterprise and SCOAP were invited to the meeting as producers of blue shrimp on Guam. Several media contacts were also invited by GADTC staff to attend the meeting.

Results

Guests began to arrive at 2:30 and continued to trickle in throughout the afternoon. Some stayed the entire time, but many stopped in only briefly. An estimated 100 people attended the meeting during the course of the afternoon, most of them as representatives of restaurants. George Tsai and Rafael Fuentes of IAE were present, but SCOAP was not represented.

Guests were greeted at the door by Mr. Hutchison and the Chianti staff. The display of prepared dishes, wine and coffee was very attractive and appetizing. The culinary offerings were mouth-watering, and the atmosphere was elegant and friendly. At about 3:30, Mr. Hutchison made a brief welcoming statement to invite everyone to sample the displayed products. There was no opportunity to make a statement to the entire group about locally raised shrimp or other aquaculture products.

However, conversation between participants was lively and congenial. Discussions with many of the attendees revealed that most were aware that shrimp were being cultured locally, but were not aware of the scale of production or the product availability. Several chefs were introduced to George Tsai, which will hopefully lead to the establishment of new customers.

The shrimp were delicious in all of the preparations. Many of the participants indicated during conversation that the shrimp were an excellent product, and that local aquaculture was a very good approach.

The most concrete results from the event were that the Japanese chef from the Hilton indicated that he would like to purchase fresh shrimp when their new restaurant featuring seafood opens, which should be very soon.

Bruce Grasley also announced his decision to feature blue shrimp every Sunday for the next year. His intention is to draw customers into the Chianti on what is normally a slow day by advertising the fresh shrimp. He also reiterated his strong interest in helping local aquaculture farmers reach the restaurant market in any way that he can. He feels that using high quality, local products will greatly benefit the restaurant industry.

The event presented an opportunity to learn more about the chefs concerns with the quality of the shrimp, and an informative discussion ensued at the close of the meeting. Since the harvesting, handling, and delivery of the shrimp had been done at the GADTC under controlled conditions, and the shrimp had been cooked within minutes of delivery, there was an opportunity for the chef to provide a first-hand evaluation of fresh shrimp quality.

Bruce pointed out that the shrimp had a range of quality differences, as could be seen in the color differences of their hepatopancreases after cooking. He said that, although the shrimp are fine if used immediately, they have a short shelf life because the head region soon imparts a bitter flavor to the shrimp.

Bruce contended that the reduced quality was due to improper harvesting and handling. Since the harvesting had been done that morning at the GADTC hatchery using standard chilling and icing procedures, it was not clear if the harvesting/handling was the issue, or if the reduced quality had to do with another factor.

Bruce also mentioned that some of the shrimp from the farm ponds have very dark or black gills, which create similar shelf life problems, and cause chefs and customers to wonder about the freshness of the shrimp. He said that blue shrimp have a very translucent carapace after they are cooked, more so than other shrimp species, which accentuates the color of the gills and hepatopancreas.

Reporters from KUAM TV, Guam Cable TV, Glimpses Publishers, and TV Guam were all present to do stories on the fresh products at the Oyster Party. Shrimp received only minor exposure through the resulting media items.

The meeting drew to a conclusion at about 5:30, with the Chianti management thanking everyone for attending.

Summary and Recommendations

The Oyster Party, although organized by a food wholesaler to showcase a wide spectrum of products, was a very good venue for drawing attention to the freshness and high quality of locally farmed blue shrimp. New interest in shrimp and aquaculture products was generated among chefs and the general public, and steps were taken toward resolving processing/handling issues.

Chefs at leading restaurants continue to search for fresh seafood products. As the concepts and themes of the restaurants change in an effort to remain competitive and attract new clientele, it is important to regularly remind chefs of the availability of locally cultured shrimp and other aquaculture products.

Invitations to the media help to raise awareness in the general public of the availability of local aquaculture products in the marketplace. They also maintain familiar media contacts which lead to improved exposure in the future. In addition, positive media attention contributes to healthy public relations for the GADTC hatchery and the Department of Commerce.

The quality of the shrimp that reach the market is known to be of great concern. The experiences of this meeting clarified that quality is a complex issue involving many inter-related factors, including but not limited to the shrimp species, production methods, harvesting methods, chilling techniques, transport methods, in-restaurant handling, and culinary preparation. Further information and local research are urgently needed to address the issue of farmed shrimp quality.

The opportunity to transfer information about shrimp and local aquaculture at this meeting was not fully taken advantage of, due to the format of the event as organized by James Hutchison of Pan Pacific. Mr. Hutchison wanted to maintain a casual atmosphere where attendees could mix freely, try the displayed products, and talk to the representatives offering those products. In keeping with this relaxed approach, no formal talks or presentations were scheduled.

While this format worked nicely for an established wholesaler like Pan Pacific, it did not seem as appropriate for entering a new market with a new product. If such an event were to be planned by the GADTC, several changes would be recommended in the organizational approach so that the meeting better suited GADTC goals. Useful information about aquaculture and the Department of Commerce role in development should be provided to attendees through brief presentations. Avenues for chefs to contact producers should be more clearly defined for attendees, and the media should be given concise information about shrimp culture to pass on to the public.

Overall, participation in the meeting was very positive, both in tangible and intangible results. Maintaining and increasing awareness of local aquaculture products leads to increased demand, new marketing opportunities, expanded sales, and a more profitable aquaculture industry. Meetings of this nature should be attended whenever possible.

APPENDIX IV

PROMOTION OF LOCALLY CULTURED BLUE SHRIMP IN RESTAURANTS ON GUAM

Promotion of Locally Cultured Blue Shrimp in Restaurants in Guam

TECHNICAL REPORT NO. 011

July 1993

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OF THE

GUAM AQUACULTURE DEVELOPMENT AND TRAINING CENTER

DEPARTMENT OF COMMERCE
Government of Guam
6th Floor ITC , Suite 601
590 South Marine Drive
Tamuning, GU 96911

PROMOTION OF LOCALLY CULTURED BLUE SHRIMP IN RESTAURANTS ON GUAM

Jerry Gardner and Dave Landkamer

June 1993

Introduction

The successful emergence of commercial blue shrimp culture on Guam in 1992 created an opportunity for aquaculture farmers to enter entirely new markets beyond their traditional direct retail outlets. Restaurants were an ideal preliminary target for market expansion with locally raised shrimp because their strong interest in the new product had already been identified.

Entering the restaurant market, however, proved to be somewhat more difficult than anticipated. Initial studies indicated that the demand was complex in terms of shrimp size, product form, price structure, and customer service (Landkamer, 1992; Landkamer and Gardner, 1993). While restaurant chefs were very interested in using locally cultured shrimp, they were very particular about the specific attributes of the product they wanted from the farmers. The most important characteristics were product quality, size consistency, and continuity over time.

At the same time, newly established shrimp farmers were concerned about production from their ponds, and had little interest in incurring additional time and expenses in marketing shrimp. Farmers could sell fresh shrimp direct to consumers without size sorting, product continuity, or added sales effort. They were reluctant to increase their costs to expand into the restaurant market, as lucrative as it might be. Therefore, farmers were not willing to make the effort to satisfy restaurant needs in terms of product form and delivery. As a result, sales to restaurants were sluggish following preliminary marketing efforts initiated in 1992.

By the end of 1992, however, it became apparent that the volume of fresh shrimp sold through the direct retail market was beginning to level off as it approached the 4,000 pound per month mark. Farmers realized that expanding the demand for shrimp would require some sort of marketing effort targeted at developing existing or new markets.

Purpose

The overall objective of the Guam Aquaculture Development and Training Center (GADTC) is to support the expansion of local aquaculture. The specific goal of this project was to bridge the gap between producing shrimp farmers and the restaurant market on

Guam. By increasing communication between the two groups, it was hoped that both sides would realize the mutual benefits of doing business together. This would allow aquaculture on Guam to enter an entirely new market and expand production.

A secondary goal was to increase awareness in the general public about the availability of fresh, high quality, locally raised shrimp. The high visibility of top local restaurants provided an excellent vehicle for meeting this goal.

Methods

Blue shrimp for the project were hatched at the GADTC and raised in a 214 m^2 Swedish pond to a size suitable for the restaurant market (18 to 30 grams each, 15 to 25/pound).

Three restaurants were selected as targets for participation in the shrimp promotion. In order to choose which restaurants were best suited to the goals of the project, the market was first divided into sectors based on primary clientele served and other characteristics. The restaurant market sectors identified for the purposes of this study are described in Table 1.

Restaurants were then placed into the different market sector categories based on their characteristics. Many fell into obvious categories, while others fit partially into two or more of the categories.

The different restaurant sectors were reviewed to determine which best served the needs of the project. The High Profile restaurant sector was chosen for the following reasons:

1) Restaurants in this sector were highly visible because of location and advertising and would be in the best position to effectively promote shrimp,
2) A large percentage of the clientele were local customers who would appreciate the high quality of the locally cultured shrimp *and* contribute to continuing demand as return customers and through word of mouth advertising,
3) The relatively high cost of the fresh shrimp, compared to frozen substitute products, would be acceptable to the restaurant for a superior product, and
4) This sector was viewed as the most likely to continue to use shrimp from local farms after completion of the promotion.

Of the restaurants in the High Profile sector, three were selected for participation in the project based on past interest in locally raised shrimp, enthusiasm to participate in the project, and willingness to conduct the desired advertising.

Table 1. Restaurant Market Sectors on Guam

Restaurant - Market Sector	Characteristics	Primary Clientele
Tourist	Located in or near resort hotels, especially on Tumon Bay; Wide variety of specialties; Expensive	Tourists, mostly Japanese; some local & business
High Profile	Advertise frequently; Elegant; Convenient location; Broad menu selection; Expensive tourists	Affluent, local business, and
Ethnic	Feature ethnic cuisine; Chamorro, Chinese, Filipino, Indian, Italian, Japanese, Korean, Thai, Vietnamese, etc.; Inexpensive to expensive	Business and families, couples tourists
Coffee Shops	Lighter meals, especially breakfast and lunch; Convenient location; Inexpensive	Business and local
Family	Broad menu selection; Inexpensive to moderate	Families and business
Convenience	Fast food; Burgers, chicken, pizza, etc.; Sit down or take out; Inexpensive	Families and business
Lounges	Food served to augment beverage offerings; Sort menu; Inexpensive to moderate	Local, singles and couples, tourists

Each restaurant was supplied with about 50 kilograms of fresh shrimp, adjusted to their particular needs. The shrimp were harvested and delivered fresh to each restaurant, according to a schedule arranged with the chef.

In exchange for receiving the shrimp free of charge, the restaurants were required to run some type of mass media advertising to draw attention to the local aquaculture product. Restaurant management was given guidelines regarding key words that should appear in the advertising. Table top display tents that could be set on each table were designed, printed, and distributed to the three participating restaurants to provide point of sale support.

The restaurants were given the freedom to decide how to offer the shrimp. Since a limited quantity of shrimp would be supplied, it was suggested that the restaurants run a special feature or add-on to their regular menu. The low cost of the shrimp (free plus their advertising costs) would also allow them to set a special

price if they chose to do so. Restaurants were informed that additional shrimp beyond those provided in the promotion could be purchased from a local farmer.

During and after the promotion, chefs were contacted by phone and in person to request their feedback on the fresh blue shrimp. In addition, shrimp presentation and flavor was casually evaluated at each restaurant by GADTC staff during the course of the project. The impact of the promotional effort on the awareness of locally cultured blue shrimp was not measured directly.

Results

The three restaurants chosen were the Chianti, the Genji, and the Islands Fisherman/Kamayan. The Chianti Restaurant, located in the Sunroute Hotel overlooking Tumon Bay, is a popular, new restaurant featuring fine pastas and nouveau Italian cuisine. The executive chef, Bruce Grasley, had been very supportive of recent shrimp marketing efforts and local aquaculture products. He is also the President of the Micronesia Chef's Association.

The Genji Restaurant is a teppanyaki style Japanese restaurant located in the Hilton Hotel on Tumon Bay. The Hilton was one of the first resorts on Guam with a long history of catering to local and tourist clientele, and often features well advertised lunch and dinner specials.

The new Islands Fisherman/Kamayan is actually a pair of restaurants under the same roof patterned after a popular establishment in Manila. Live shrimp, lobsters, and fish are held in viewing tanks, and the restaurants specialize in traditional Filipino cuisine and ultra-fresh seafoods.

For the Chianti and the Genji, shrimp were collected from the pond and chilled in iced sea water for 15 minutes. They were then placed into coolers layered with ice and delivered immediately to the restaurants. The Islands Fisherman/Kamayan received live shrimp for their tanks through a special arrangement with Inarajan Aquaculture Enterprise (IAE). IAE had the necessary equipment to deliver live shrimp, while the GADTC did not.

The three restaurants received shrimp and ran their specials during slightly different, overlapping time periods that cumulatively spanned about five weeks, from April 13 to May 14. A list of shrimp delivery dates is provided in Table 2.

Each of the three restaurants utilized a different approach in offering the shrimp to their customers. The Chianti created several special menu items featuring shrimp that were offered as a weekend special at $13.00 each (about 35% off usual prices). The Genji offered five special preparations of shrimp in the usual menu

price range. The Islands Fisherman used their existing menu, but with reduced prices (50% off) for entrees with shrimp.

Table 2. Shrimp Delivery Dates to Restaurants

Restaurant	Ran Shrimp Specials	Delivery Date	Kgs.
Chianti	April 14 - 18	April 13	12.4
		April 15	11.6
		April 18	7.3
Genji	April 30 - May 14	April 29	14.8
		May 5	14.7
		May 10	15.7
Is. Fisherman	May 5 - 9	May 4	45.4
		May 7	11.4

All restaurants produced highly visible advertisements for both the radio and newspaper that ran at the time of their specials.

Table top promotional tents were designed and printed by the graphics art firm of Tanner and Tanner Design Inc.. The free standing tents measured 4 inches wide by 6 1/4 inches tall, and had attractive shrimp graphics overlaid with text about the fresh shrimp (Appendix 1). They were placed on restaurant tables to provide point of sale information to customers, and encouraged them to try the local blue shrimp on the menu.

At the beginning of the project, the author and Chef Bruce Grasley from the Chianti Restaurant were guests on the popular John Anderson K57 radio talk show to discuss the arrival of locally farmed shrimp at Guam restaurants, and alert listeners to the upcoming shrimp specials. This provided an effective means of informing the public about aquaculture. A transcript of the talk show interview is provided in Appendix 2.

All of the participating restaurants were visited to sample their preparations of shrimp during the time they ran their specials. In every case, the shrimp were excellent in appearance and taste as expected.

The chef's response to the blue shrimp was very positive as expected, reinforcing the results from previous product evaluations. The chefs from all of the restaurants agreed that the shrimp were an excellent product to work with. Its freshness and taste was rated highly above its frozen counterpart. It was also reported that the customers were very pleased with the flavor and overall quality of the shrimp, and were surprised to learn that they were grown on Guam.

overall quality of the shrimp, and were surprised to learn that they were grown on Guam.

All three restaurants sold out of shrimp during their specials, indicating that the response to their specials exceeded their expectations. A very encouraging positive outcome of the project was that all three restaurants elected to continue using locally raised blue shrimp on their menus. Chefs were placed in direct contact with farmers and initial deliveries were facilitated.

The outcome of this marketing project went beyond original expectations. The objectives of informing the public about aquaculture on Guam and creating a marketing link between local shrimp farmers and restaurants were achieved. That all three of the restaurants decided to continue using locally farmed shrimp in their menus indicates the value of well planned marketing efforts and the potential for future expansion of shrimp sales.

Since this marketing project was much more successful in securing commitments from restaurants to buy shrimp than previous efforts, it may be that customer input is the driving factor. Previous marketing work introduced the shrimp to the chefs, who universally agreed that the product was excellent, but did not follow through with purchases. Supplemental customer approval of locally farmed, high quality shrimp may have been a decisive factor in the chef's decisions to continue using the product. This theory is worth keeping in mind for future marketing efforts.

With the addition of the new restaurant orders, present market demand for locally cultured shrimp exceeds current production. This is partly due to the fact that only one farm is producing enough shrimp to be able to market it on a continuous basis. As a result, the opportunity to move ahead with additional expansion into the restaurant markets, or other potential markets, is constrained. In order for this to change, shrimp production must increase, either from existing or new aquaculture farms.

References

Landkamer, D.J. 1993. Fresh Shrimp Showcase Report. GADTC Report.

Landkamer, D.J. 1992. Preliminary Introduction of Locally Raised Shrimp to Hotels and Restaurants on Guam. GADTC Report.

APPENDIX I

The Islands CATCH

Enjoy the freshest produce of the sea. Pick your choice from a whole range of live and fresh seafood. Have it cooked any way you want. It can only happen at the Islands Catch, the seafood market section of Islands Fisherman. A dining experience that is quite unlike any other.

50% OFF on LIVE, FRESH BLUE SHRIMP

(Limited Supply) Farm-Raised & Locally Grown.

The ISLANDS FISHERMAN

The Best Seafood From The One Who Knows Seafood Best

Business Hours: Monday thru Sunday—Lunch: 11am to 2pm; Dinner: 6pm to 10pm. We welcome private functions; company meetings.

A Passion for Perfection

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Tel. 646-6968•71•76•78•80 — Fax 646-6989

Blue Shrimp Special

Well the award winning Chefs at the Hilton have done it again. In cooperation with our own Department of Commerce and local Entrepreneurs, The Genji will be offering one of the worlds most exotic dishes found right here on Guam.....Blue Shrimp. Six delicious recipes will be available for your selection from May 1-7 If you are a connoisseur of shrimp, you wont want to miss this event.

Blue Shrimp Tempura
$15.00
Blue Shrimp wrapped in Bacon Teppan Yaki
$17.00
Blue Shrimp and Sirloin
$25.00
Blue Shrimp and Tenderloin
$25.00
Fresh Blue Shrimp
$20.00

Date : May 1-7, 1993
Venue : Genji
Time : Lunch & Dinner
Price: See Above

* Plus 10% ServiceCharge

Chianti
FINE PASTA

PRESENTS

WEEKEND SHRIMP FEST

Featuring locally farm raised Blue Shrimp, Saturday, Sunday & Monday, June 19-21 1993 • WHILE SUPPLY LASTS

Featuring

APPETIZERS:

Shrimp Cocktail or Shrimp Scampi **$6.95**

COMPLETE DINNERS:

(CHOICE OF ONE)

• Shrimp Florentine • Shrimp Milanese • Garlic Shrimp
• Spicy Shrimp with Avacados and Fettuccine

All Dinners Include: Bread and Butter Service, Minestrone Soup, Caesar Salad • **$19.95**

JOIN US DAILY FROM 7:00 - 10:00 AM FOR BREAKFAST AT *CHIANTI*

LOCATED LOBBY LEVEL SUNROUTE HOTEL TUMON
646-2906 FOR RESERVATIONS

Chianti
FINE PASTA

PRESENTS

CHIANTI'S SHRIMP FEST

Featuring locally farm raised Blue Shrimp Saturday June 26, Sunday June 27 & Monday June 28, 1993• WHILE SUPPLY LASTS

FEATURING

SHRIMP PUTTENESCA
Spicy Tomato Suace with Capers, Onions and Hot Peppers

SHRIMP AND RED PESTO
Roasted Red Peppers, Garlic, Cheese, Sun Dried Tomatoes and Walnuts

SHRIMP SCAMPI
Sauteed in Butter, Lemon and Garlic

SHRIMP W/LINGUINE
and Clam Sauce

All Dinners Include: Bread and Butter Service, Minestrone Soup, Caesar Salad • $19.95

JOIN US DAILY FROM 7:00 - 10:00 AM FOR BREAKFAST AT *CHIANTI*

LOCATED LOBBY LEVEL SUNROUTE HOTEL TUMON
646-2906 FOR RESERVATIONS

Chianti
FINE PASTA

BACK BY POPULAR DEMAND

CHIANTI'S SHRIMP FEST

Featuring locally farm raised Blue Shrimp
Saturday May 30 & Sunday May 31, 1993
WHILE SUPPLY LASTS

FEATURING

SHRIMP PUTTENESCA
Spicy Tomato Sauce with Capers, Onions and Hot Peppers

SHRIMP AND RED PESTO
Roasted Red Peppers, Garlic, Cheese, Sun Dried Tomatoes and Walnuts

SHRIMP SCAMPI
Sauteed in Butter, Lemon and Garlic

SHRIMP W/LINGUINE
and Clam Sauce

All Dinners Include: Bread and Butter Service, Minestrone Soup, Caesar Salad • $19.95

JOIN US DAILY FROM 7:00 - 10:00 AM FOR BREAKFAST AT *CHIANTI*

LOCATED LOBBY LEVEL SUNROUTE HOTEL TUMON
646-2906 FOR RESERVATIONS

Chianti
FINE PASTA

WEEKEND SHRIMP FESTIVAL
FRI. SAT. SUN. APRIL 16-18

1) SHRIMP & RED PESTO
2) SHRIMP SCAMPI
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Grown locally in aquaculture ponds guarantees that Guam's blue shrimp are the freshest possible shrimp our chefs could choose for the variety of delicious recipes you'll find on our menu.

We entreat you to try them, to ask for the Guam blue shrimp. And discover that the true color of fresh taste is undeniably blue.

Local shrimp culture is supported by the Guam Department of Commerce. For more information, call the GADTC hatchery at 734-3011.

Tasum & Tasum Diaso Inc.

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The True *Color* Of Fresh *Taste*

Tasum & Tasum Diaso Inc.

Final Report

Pacific Aquaculture Association

Preliminary Spawning and Larval Culture Trials for Groupers from Guam and Palau

By: William J. FitzGerald, Jr., Michael Bauerlein, and Clyde S. Tamaru*

TECHNICAL REPORT NO. 018

January 1994

TECHNICAL REPORT SERIES

OF THE

GUAM AQUACULTURE DEVELOPMENT AND TRAINING CENTER

DEPARTMENT OF COMMERCE

Government of Guam

6th Floor ITC , Suite 601

590 South Marine Drive

Tamuning, GU 96911

PACIFIC AQUACULTURE ASSOCIATION

PRELIMINARY SPAWNING AND LARVAL CULTURE TRIALS FOR GROUPERS FROM GUAM AND PALAU

FINAL REPORT

Prepared by

William J. FitzGerald, Jr., Mike Bauerlein and Clyde S. Tamaru*

January 1994

Guam Aquaculture Development and Training Center
Department of Commerce
Government of Guam

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Special thanks are extended to Clyde Tamaru for his assistance during the project and to all of those individuals that contributed to the success of the project. The assistance of Annie Orcutt of the Pacific Aquaculture Development Program, especially in regard to expediting the necessary arrangements for Clyde Tamaru's participation, is greatly appreciated. The assistance of the Micronesian Mariculture Demonstration Center (MMDC), Department of Natural Resources in providing facilities and staff support in Palau is greatly appreciated. Special acknowledgement is made of the contributions by Gerald Heslinga, David Idip, Don Hanser, and Becky Madraisau of MMDC. The assistance of Mr. John Gibbons, State Executive Administrator of the Koror State Government facilitated the permitting of the collection of groupers from the Ulong Channel reserve. The assistance and support to the project provided by Nancy Wong in facilitating the process through some of the bureaucratic steps in the implementation of the project is greatly appreciated. The extraordinary fishing expertise of Thomas Taro of Palau was instrumental in the success of the project.

PACIFIC AQUACULTURE ASSOCIATION
PROJECT REPORT

FINAL REPORT

PROJECT TITLE
Preliminary Spawning and Larval Culture Trials of Groupers from Palau and Guam

PERIOD COVERED BY THIS REPORT
July 1, 1992 thru December 31, 1993

GRANT PERIOD
July 1, 1992 thru December 31, 1993 (extended to June 1994)

PRINCIPAL INVESTIGATOR
William J. FitzGerald
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Guam Department of Commerce
590 South Marine Drive
Tamuning, Guam 96911

Mike Bauerlein
Guam Aquaculture Development and Training Center
Guam Department of Commerce

PROJECT OBJECTIVES
The project consists of the following objectives in the preliminary work on groupers:

- To spawn selected species of groupers in Palau and Guam.
- To ship viable grouper eggs from Palau to Guam for larval culture.
- To conduct preliminary larval culture procedures adapting proven methods for a selected species from Palau and Guam.
- To apply developed larval culture procedures to potential aquarium species.
- To establish preliminary guidelines for developing grouper spawning and larval culture in the region for an indigenous species.

SUMMARY OF FINDINGS

Four species of groupers (Plectropomus areolatus, Epinephelus fuscoguttatus, E. microdon, and Cephalopholis miniata) were utilized for the investigation into the spawning and larval culture. Three species were targeted as food species with the fourth, Cephalopholis miniata, targeted as an aquarium species. Epinephelus microdon was used as a model species for conducting the spawning, egg development, hatching, and larval rearing work to obtain detailed information. Similar work was carried out for E. fuscoguttatus, but with less detailed data collection.

Maturation of E. microdon was induced with a two injection protocol (priming and resolving) of HCG. The injection protocol was refined with injections at 700/1400 IU/kg for the 1st and 2nd injections respectively. The average production of fertilized eggs per fish per spawn was 1.13×10^6. The length x weight relationship, size frequency distribution, sex composition by size, size at sexual transition from female to male were identified. Characteristics of sperm activation and extension was documented. Embryonic development was documented photographically. Characteristics of spawned eggs (fatty acid profile) and optimum egg density (up to 20,000 eggs/l) for transportation from Palau to Guam were identified. Initial feeding trials compared cryopreserved oyster trochophores, trochophores with rotifers, size selected rotifers and the control of no feed, which resulted in no significant difference in treatments; however, the results indicated an improved survival with oyster trochophores. Intensive (20 larvae/l) and extensive (2 larvae/l) larval rearing was conducted with larvae reaching day 6 and 15 for intensive and extensive respectively before termination due to high mortality.

Transportation procedures for adult live groupers was developed. Successful transportation of E. microdon (6) and C. miniata (44) was carried out to establish initial broodstocks at GADTC.

METHOD

There were no collections of groupers attempted in Guam, since the results of the grouper resource survey in Guam at the end of the previous project indicated an inadequate population for the reliable collection of spawners. Therefore, all of the collection efforts were focused in Palau.

Spawning

The fish were caught by hand-line while bottom fishing at known aggregation channels. Fish were maintained on-board the boat in live bait wells. They were transported back to the Micronesian Mariculture Demonstration Center (MMDC). The fish were then transferred to 2,000 and 4,000 l tanks at MMDC. Water flow and aeration were provided. Groupers that had an inflated gas bladder were deflated using a hyperdermic needle. At MMDC the fish were held in tanks and underwent induced spawning procedures utilizing Human Chorionic Gonadotropin (HCG).

The spawning procedure and results are provided in Appendix IX.

Shipping Eggs

Eggs were shipped at a density of 5,000 eggs/l to Guam for hatching at GADTC. Shipment density trials were carried out at MMDC to determine an optimum density considering egg viability and quantity shipped. The trials simulated the shipping condition and duration. A range of 1,000 to 100,000 eggs/l was considered. Appendix IX elaborates on the procedures used in the trial.

Hatching/Larvae Culture

Incubation of the eggs goes through four stages (initial stocking of eggs, separation of eggs, quantifying the eggs, and stocking in larval rearing tank). Eggs are stocked in the incubation tank with aeration to keep the eggs suspended, with a water flow exchange at a rate of 100%/day. Separation of viable fertilized eggs and dead eggs is carried out in the incubation tank by turning off the aeration and allow the eggs to separate. The viable eggs float to the surface. Increasing the salinity to 40 ppt assists in the separation. The viable eggs are skimmed off and transferred to 100 l tank. The eggs are quantified in the 100 l tank through replicate 100 ml samples. After quantification, the eggs are transferred to the larval rearing tank at the desired density. Volumetric transfer from the quantification tank assures proper stocking density.

The larvae culture procedures are identified in Appendix IX.

RESULTS

Collection I

Two GADTC staff members (Mike Bauerlein and Mark Norman) traveled to Palau on March 15, 1993 for the initial spawning work. Preparations were made at GADTC to accommodate the pending first shipment of grouper eggs from Palau. Hatching tanks and larval rearing tanks along with the biological filters were set up for receiving the shipment. Coordination with Palau counterparts including MMDC, private fishermen, and the Koror State Government was carried out in advance of travel to Palau so that arrangements were made for the project. Materials and supplies necessary for the spawning work in Palau were obtained and organized. The first two days in Palau were devoted to obtaining the necessary permits and finalizing arrangements with the fishermen and tank space at MMDC. An initial fishing trip was made on March 17, 1993. The results of the fishing and subsequent spawning attempt are attached as Appendix I.

The catch was poor, since GADTC staff were not permitted to fish in the aggregation channel. This resulted in an inadequate number of spawners. The one female and three males were ripe. The fish were in poor condition, since the catch was bloated (swollen air bladder) from fishing in deeper waters away from the aggregation channel. Because of the inability to obtain permission to fish in the aggregation site, as done in the previous year's project, the GADTC staff terminated the trip early. As a result, the intended spawning work could not be carried out on this trip and also resulted in the loss of future months spawning work until the issue could be resolved. Further substantial negative impacts on the project included the elimination of replicate treatments and elimination of the intended original main target species - Plectropomus areolatus. These problems arose despite thorough efforts through the project

proposal preparation stage and prior to implementation to assure full coordination with Palau.

As an alternative to Palau, if the above issues could not be resolved, contacts were made with Pohnpei's Department of Conservation and Resource Surveillance to determine the possibility of conducting the work in Pohnpei. The initial response was very favorable and they were very excited about working with us on this project. However, this would have substantially increased the work and introduced a number of new uncertainties as opposed to continuing the work in Palau where a significant amount of previous work had been accomplished (PAA Grouper Year I) in establishing the base of this current project. Unfortunately, according to information from Pohnpei their spawning season was over for the year for the main target species.

Resolution of Permit and Collection Issues

The lead PI traveled to Palau in early May to resolve permit problems in Palau that arose during staffs' trip in March. There was a resistance to allow fishing in the Ulong channel which is a reserve. Permits had been issued for research purposes to carry out the previous grouper project.

Meetings were held with various Palau officials to resolve some permit problems and to obtain permission to fish in reserves for groupers. A meeting was held with David Idip, Director of Natural Resources, to obtain permission to fish for groupers in the reserve to carry out the PAA project. In addition, a scientific research and collection permit was requested for the upcoming collection in May. Both of these objectives were successfully carried out. A meeting was held with John Gibbons, Director, Koror State Government, to obtain a modification in the previous permit received to allow for collection of groupers within the reserve channels. This issue was also successfully resolved.

With the resolution of these major barriers to implementing the project, the project could proceed as originally planned (with the exception of lost opportunities for spawning of Plectropomus sp., since the season was nearly over). The recent passage of new legislation in Palau banning the export of fish for a four month period (March - June) further complicated the issue; however, it was clarified that the ban does not apply to research work.

The owner of a private aquarium fish collection business was not in Palau, so arrangements for the collection of aquarium groupers could not be carried out.

In addition to resolving the permit issues, arrangements with the private fisherman that assists on the project was made for the next trip by GADTC staff to Palau. Tank space at MMDC, and room and car arrangements were similarly made. Arrangements for Guam Aquaculture Development and Training Center staff to travel to Palau to continue the project were also made.

Collection II

Mark Norman and Jerry Gardener of GADTC were in Palau from May 16 to May 21, 1993 to attempt to spawn Plectropomus areolatus. The two fishing trips to the Ulong Channel reserve resulted in adequate fish for spawning trials. Appendix II provides the details of the collection and the resulting spawning attempts. Fish were stripped spawned from the first collection day; however, they were not fertilized. The spawning of the second group was curtailed when

staff discovered that the two intended males to be used were missing. A report from the MMDC staff informed us that they had died that day.

New problems were encountered with permits. This included the issuance of a Certificate of Origin. Staff were referred to the Conservation Office for the issuance of a certificate. However, Mr. Dmier O'Tobed refused to issue the permit regardless of all of the permits issued for the project to collect the groupers for research. This was in reaction to the new legislation that banned the export of all marine species during March to June. With the assistance of various contacts in Palau the issue was resolved with the intervention of the President of Palau; however, by the time the Certificate of Origin was issued it was too late to ship the grouper eggs.

Difficulty was also encountered in the logistical arrangements for the shipping of eggs. The expansion of the tuna transshipment activity in Palau had blocked off most of the cargo space on certain flights. This made scheduling shipments more difficult.

A June trip to Palau to spawn Plectropomus sp. was cancelled due to the continued permit problems that GADTC staff were faced with.

Collection III

Arrangements were initiated in the later part of June to contract the services of Dr. Clyde Tamaru (C's Consultants Hawaii) to assist in the spawning and larval culture of groupers. A work plan was developed and submitted to the Pacific Aquaculture Development Program. The scheduled commencement of the consultancy was July with the assistance of PADP staff in expediting the necessary paper work. The work in Palau for the remainder of the project focused on Epinephelus fuscoguttatus and E. microdon, since the Plectropomus sp. spawning season ended in June. In addition, work would be initiated on the species of groupers targeted for the aquarium trade.

The lead PI headed the next trip to Palau to continue the project in July. This was to make sure that the logistics of the project were fully carried out and ensure the maximum effort to resolve possible hindrances of the project. A subcontract with Dr. Clyde Tamaru was implemented in July 1993. Dr. Tamaru accompanied the lead PI on the following two collections in Palau during July and August. The collection data is presented in Appendix III. The objectives of the project were successfully carried out during this trip. Details on the results are presented in the GADTC Technical Report No. 16 (Appendix IX).

Since the spawning season of P. areolatus was over the target species were E. fuscoguttatus and E. microdon. In addition, a shipping trial of live adult E. microdon was carried out in Palau. The results (Appendix V) indicate that live adults can be successfully shipped. Utilizing this procedure, the shipment to Guam of the smaller live adult E. fuscoguttatus collected was carried out. Results of this shipment are presented in Appendix VI.

An evaluation of potential candidate aquarium species of groupers was carried out. Seven species of groupers collected during the July trip offered some potential as aquarium species. Details of the collection and the evaluation are presented in Appendix VII. The three top priority species were Cephalopholis miniata, C. urodeta, and C. spiloparaea.

Collection IV

The lead PI and Dr. Kamaru made a final trip to Palau in August. This is the last month of the spawning season for the targeted E. fuscoguttatus and E. microdon. Work was focused on E. microdon as the model species. Observed spawning behavior of E. microdon was documented during this trip (Appendix VIII). A final report of this trip and the previous one with the results of the work are attached as Appendix IX. All of the project objectives were carried out in Palau with the gathering of a substantial amount of data beyond the original intent of the project. This data provides a very strong foundation to the refinement of spawning, egg incubation, egg development, hatching, and initial larval stages. This will substantially facilitate future work with groupers.

To establish a broodstock of C. miniata in Guam to facilitate future work on the maturation, spawning and larval rearing of this species, 35 adult fish were shipped to Guam. This was successfully carried out following the shipping procedures developed in Appendix V. On arrival at the Guam Aquaculture Development and Training Center the fish were held in 2,000 l tanks and treated over a two week period with antibiotics (Prefuran) to reduce potential infections from the shipping and handling. The broodstock was divided between a 20 m-ton fiberglass tank and a 26.8 m^2 concrete tank. The fish exhibited minimal aggressive and territorial behavior in the smaller 2,000 l tanks under crowded conditions. However, aggressive territorial behavior was very apparent in the larger tanks. Various degrees of damaged fins resulted from the aggressive behavior. Some fish lost major portions of dorsal and caudal fins. The damaged fish were removed from the larger tanks, received antibiotic bath treatments and were held separately until they recovered. Seventeen fish died as a result of the aggressive behavior. Both of the larger broodstock tanks were divided into sections. A mated pair was placed in each section with the intent of reducing the aggressive interaction and stimulating mating within the mated groups. Monthly cannulation of the broodstock was initiated to collect data on maturation. An egg collector was placed in each tank to monitor spawns. This specific long-term maturation monitoring of C. miniata was not part of the current project; however, the work will continue past the completion of current project.

DISCUSSION OF RESULTS

E. fuscoguttatus is a large species reaching a weight of over 15 kgs and not as common as E. microdon. Therefore, E. microdon was utilized as the model species for the more detailed information collected and trials conducted. This work included the following.

- Assessment of reproductive biological characteristics
- Induction of spawning
- Spawning behavior
- Temporal changes of grouper oocytes during final maturation
- Fatty acid profiles of spawned eggs
- Activation of grouper sperm at various salinities
- Sperm extenders
- Embryonic development of grouper larvae
- Egg transportation
- Effect of light on newly hatched larvae
- Larval rearing
- Live feed options
- First feeding experiments

- Intensive larval rearing
- Extensive larval rearing
- Effect of formalin on survival of live feeds

As mentioned, the first two collections in Palau did not result in eggs being shipped to Guam for the larval rearing phase due to the various complications mentioned. However, the last two trips were very successful in accomplishing all aspects of the work in Palau and in shipping eggs to Guam. The protocol for the larval rearing trials and arrangements were made in advance of the travel to Palau. This included obtaining cryopreserved oyster trochophores as part of the initial feed trials. Details of the larval rearing trials are included in Appendix IX. Extensive larval rearing trials were carried out in addition to the more controlled tank based intensive larval rearing. The results and discussion are included in Appendix IX.

CONCLUSION

The key problems encountered in implementing the project were the following:

- The constraint in obtaining permission to fish within the aggregation site in Palau was a hindrance in the initial implementation of the project and jeopardized the continuation of the project.

- The new law in Palau that bans the export of marine species during March thru June caused confusion with the Palau officials on how to treat scientific research projects. This resulted in difficulty in obtaining the necessary permits to carry out the project.

- The expansion of the tuna transshipment industry in Palau with the increased number of foreign longline vessels based in Palau utilized most or all available cargo space on the commercial flights. This made the shipping of the eggs to Guam difficult.

All of the project objectives were successfully carried out despite the above logistical and procedural problems. Substantial data was collected during the duration of the project which provides a strong foundation to future work on the grouper species considered in this project. However, the difficulty in completing the larval rearing cycle remains. Future work will focus on the critical stages in the larval rearing which includes the initial feeding, and the critical points associated with days 2-3 and 6. In addition, the initial broodstocks that were established at GADTC of E. microdon and C. miniata need to be expanded. The continued utilization of E. microdon as the model species is recommended. Once key issues in the reproduction and larval rearing are refined this will facilitate adoption of procedures to the other three target species.

William J. FitzGerald
Principal Investigator

March 12, 1994
Date

APPENDIX I

COLLECTION I - MARCH 1993

GROUPER SPAWNING COLLECTION I

MARCH 1993

March 15 to 19, 1993

Participants
Mark Norman (GADTC Marine Tech II)
Michael Bauerlein (GADTC Biologist Supervisor)

Moon Phases
Quarter Moon - March 15?
New Moon - March 23

First Day's Catch

Date:	March 18, 1993
Location: of the Ngerumkaol Reserve	Barrier Reef, approximately one mile southwest
Depth:	30 to 40 feet
Tides:	Fished during the outgoing tide
Fishing time:	1050 - 1350 (about 3 hours)
Fisherman:	Presley Etibek
Moon phase:	5 days prior to new moon
Travel time:	About one hour each way
Fishing method: as bait. Only one line out.	Hand line bottom fishing. Used small "sardines"
Species caught:	*Plectropomus aerolatus* (4).
Condition of the fish: free flowing milt.	The female had ripe eggs. All of the males had

P. aerolatus	Female	1170 g	died before first injection
P. aerolatus	Male	1545 g	
P. aerolatus	Male	1980 g	
P. aerolatus	Male	1985 g	

Spawning results:

There was no spawn due to the death of the female.

Palau trip report: March 15 thru March 19, 1993

Travelers: Michael Bauerlein and Mark Norman

Chronology of Events

March 15: Arrived in Palau at about 1930 local time.
Rented a car and drove to MMDC.

Met Tim Adams of the South Pacific Commission who was staying at MMDC for a few days on his way back to New Caledonia.

March 16: Checked in with Gerry Heslinga, Becky Madraisau, and David Idip (the facility director). Neither Becky or David were expecting us. David thought the grouper project was completed. He has a copy of the final report. Becky was very cooperative and designated four tanks to hold any groupers we might catch.

Filled out "Scientific Research Permit" and paid $100 on MMDC. (We were told this would greatly expedite the approval process.)

Made contact with Franny Reklai regarding charter boat reservation.

Bought supplies needed for project.

March 17: Received permit from David.
The permit forbade us to fish in the sanctuary.
We met with Franny again and he suggested we fish on the outer reef about a mile southwest of the sanctuary. He arranged for a fisherman to pick us up at MMDC the next morning to fish for grouper.

Talked with Nancy Wong at the Fisherman's Coop.
She was interested in hearing about our success with penaeids.

Paid $50 for a fishing permit from Koror state which also barred us from fishing in the sanctuary.

Phoned Dot to tell her that our permits restricted us from fishing in the sanctuary and that Bill should not fly down on Thursday night unless we called with news that we had caught fish. She informed me that Bill was not returning from Hawaii until Friday.

March 18: Discovered that we had not taken the HCG with us, so phoned Dave at the hatchery and asked him to ship it to us on the next Continental flight.
Fisherman Presley Etibek arrived at the MMDC dock at 0820. We boarded and proceeded to a shallow mangrove estuary on the east side of Koror to catch small sardines for bait. We collected about fifty baitfish. We started fishing at 1020 in the lagoon just north of the sanctuary. Presley said he had been diving there a few nights earlier and had collected grouper. We had no luck, and proceeded to the barrier reef southwest of the sanctuary. We fished for about three hours at this site on the outer reef near the sanctuary. Presley caught one female and three male Plectropomus aerolatus. Several other

fish were able to escape, and our incidental catch was two jacks (papio) and two snappers. We tried moving to shallower water close to the exposed reef, but had no luck there. All of the fish bloated enough to cause them to float upside down in the bait well. We tried using a needle to bleed the air but the water was too rough and the fish wouldn't hold still. We caught the female first, at 1130, and she was able to right herself after about twenty minutes. Only one of the males righted itself before our return to MMDC. Both Mark and I felt a little seasick due to the choppy sea conditions. We returned with the fish to MMDC. We bled off the air from the two upside down males at about 1600. The female and the other male were righted but their dorsal fins were out of the water exposed to the air. We made no attempt to release more air from these two. Mark cannulated the female and found her to be ripe. Sent a fax to Dot requesting hatchery personnel to expect a shipment of eggs on Sunday. They needed to arrange for someone from Aquatics and Wildlife to inspect the shipment upon arrival.

March 19: Found the female grouper dead in the morning. The males were OK.

Phoned Linda Sablan with the message to ignore the fax I sent and to relay the message that we could not send an egg shipment on Sunday. Also, there was no need for Bill to fly down.

Contacted Franny immediately but had no luck chartering a boat. Checked with MMDC about using their boat but none of the staff were available to take us out. Mark contacted the Fish and Fins and Blue Marlin charter boat operations to charter a boat on the 19th or 20th but they needed a week's advance notice. Mark also asked Becky if he knew a body with a boat to charter, but Becky didn't know of anyone. Picked up the HCG at the Continental office. When it appeared that chartering a boat for Saturday was questionable, we decided to return to Guam on Friday night flight. We cleared up our bills at MMDC.

I met with Gerry Heslinga and obtained one of his Customer Satisfaction Forms. He said he could sell us coolers for egg shipments at $15 each.

I met with Larry Shannon (sp?) regarding collection/spawning habits of aquarium grouper. Also talked about obtaining wrasses from him for the clam project. He said he still owes Gerry Heslinga about 300 wrasses. He also said he knew only certain species of wrasses would be allowed into Guam. He didn't sound very excited about providing us with wrasses. But he was very interested in providing aquarium grouper species for us.

List of phone numbers for future reference:

Larry Shannon fax 488-2305
home 488-1962 can be reached between 6 and 7 p.m. Guam time
Franny Reklai home 488-2863

work
Presley Etibek home 488-2873

Mike Bauerlein
Biologist Supervisor

Mark Norman
Marine Technician II

APPENDIX II

COLLECTION II - MAY 1993

GROUPER SPAWNING REPORT
COLLECTION II

by MARK NORMAN

FIRST DAY CATCH:

DATE:	May 17, 1993
LOCATION:	Ulong Channel
DEPTH:	18 - 20 feet
TIDE:	Going out
FISHING TIME:	1030 - 1300
FISHERMAN:	Thomas Taro
MOON PHASE:	Three days before new moon
TRAVEL TIME:	40 minutes each way
FISHING METHOD:	Hand line bottom fishing using sardines as bait

CONDITION OF THE FISH: First catch was one shark and five snappers. Moved to the shallower part and started catching female groupers but no males. We moved to deeper water, about 30 - 35 feet in depth, and started catching male groupers but the sharks kept taking them away. We were able to catch P. aerolatus: 3 males and 13 females. We also caught 3 E. fuscoguttatus, weighing about 10 kg each, which we released without cannulating. All the P. aerolatus females were cannulated on the boat. The eggs looked very small to me and did not separate. Males had free-flowing milt. Males and females were separated into two compartments aboard the boat. The day was sunny and hot.

SPAWNING: When we returned to MMDC, we separated the males and females into two different tanks. None of the fish had bloated. At 1600 five females were weighed and received their first injection. Their average weight was 2.2 kg. The fish were transferred into a new tank. This time we had two tanks for females. Fish were checked periodically to make sure they were alright.

On Tuesday, the fish received their second injection. On Wednesday morning at 0100, females were cannulated to check the condition of the eggs. Eggs looked good, although small, and did not separate yet. All of them were swollen. At 0200 we cannulated them again and started preparing our supplies. At 0400 one was cannulated and found that she was getting ready to spawn; we could not delay the spawning (strip) any longer if we were to meet the flight schedule. So we proceeded to strip the females. First, we added 100 ml of sea water to a beaker and put it aside, squeezed eggs into a dry beaker, and immediately squeezed the milt on to the eggs. Then we poured the 100 ml of seawater into the container with the eggs and started mixing. After the eggs and milt were mixed, we transferred them into a 10 gallon bucket for the washing or separation. First, we waited until the eggs came to the surface. Then we skimmed them into a styrofoam cooler containing 50 liters of sea water. From there, we would do our final separation into the bags. At 0800, I took

samples from each spawn and checked them with the microscope. The eggs did not look like they were fertilized. I did not see signs of development. Three hours after that I looked again; the eggs looked clear with nothing inside. After five hours I looked again. There was still no sign of development. No larvae were observed to hatch from any of the spawns.
Egg counts from two of the spawns: 400,000 and 186,000.

SECOND DAY CATCH

DATE: May 18, 1993

LOCATION:	Ulong Channel
DEPT:	25 - 30 feet
TIDE:	Going out
FISHING TIME:	0900 - 1300
FISHERMAN:	Thomas Taro
TRAVEL TIME:	40 minutes
MOON PHASE:	2 days before new moon
FISHING METHOD:	Hand line bottom fishing

The 6 males were caught from the deeper part and 10 females were caught from the shallower area, about 15 - 20 feet deep. None of the fish bloated. At this time we only caught one shark and no snappers, even though we fished in the deep part of the sanctuary. Fish were transported to the facility and placed with the 3 males caught the first day. At 1600, the other five females received their first injection. Fish were checked periodically to make sure they were alright following the injection.

On Wednesday at 1600 they received their second injection.
The weights of these five fish were 2.6, 2.2, 2.3, 2.4, and 2.4 kg.
On Thursday morning at 0100, the fish were cannulated and the eggs looked small, but separated. At 0300, the fish were swollen and ready to spawn. At 0400 we were getting ready to strip when we found out that two of our males were missing and the two that were left had no milt (possibly extracted by someone else?).

GROUPER SPAWNING REPORT IN PALAU

MAY 1993

OBJECTIVE. The purpose of the trip was to harvest grouper eggs and ship them to GADTC for hatching.

Project began on May 16, 1993, with our arrival in Palau around 2000; we collected our gear (bags and equipment) and proceeded to MMDC. Upon our arrival at the facility, (MMDC), Mark and I looked for the manager, Mr. Heslinga. When we found him we were greeted with hostile words sprinkled with profanity. We quickly separated ourselves from this individual and went to the dorm area to sleep.

At the time of our arrival we also had called Nancy Wong about the Fishing Permit for the sanctuary; she had not acquired it. The morning of May 17, we again called Nancy about the Permit, she still had not received it and indicated that we should go fishing without it. We were told by Nancy Wong and Franny that it would be OK. During that morning we also encountered Gerry Heslinga, the MMDC manager. He apologized for his inappropriate greeting but said we should have not come to his area at that time of night, which was about 2045.

Thomas, the boat operator, had been contacted early in the morning; he arrived at 0800. By 0915 we were on our way to the sanctuary. We stayed in the sanctuary for 4.5 hours; during that time we caught 13 females and 3 males. Returning to MMDC, we followed the protocol, paying close attention to the times for the fish to settle and for the first injection. The average weight of the groupers was 2.2 kg. We still had not obtained our fishing permit by the end of the day.

Tuesday, May 18: We went fishing again in the sanctuary without a permit, leaving the facility at 0800. Fishing time was 5 hours. Six males were brought back; we now had 9 males. I had also inquired of Gerry about the Certificate of Origin that would be needed in order to ship the eggs; he indicated that he could arrange that. By early Wednesday morning that had changed and Gerry said we would need to meet with the Chief Conservation Officer, who was not pleased with this project.

Again on Tuesday, we followed the project procedure, weighing, injecting and separating the groupers as specified.

Wednesday, May 19: We begin at 0100 the procedure to collect and fertilize the eggs. We had also encountered another problem with shipping: there wasn't any room on the morning flight. The eggs would have to stay at MMDC until 1600. As I already mentioned, we still did not have the Certificate of Origin. We have reason to believe that the President of Palau intervened on our behalf to have the Certificate of Origin granted. We were called to the office of Demier O'Tobed, Chief Conservation Officer, and told that we were breaking the law of Palau. This was not the legal time to export fish and whoever gave us the permission to do so was also breaking the law. The Certificate was not issued until 1530. We had been detained long enough for the schedule to fall apart. There was no way to return to MMDC in time to make the shipment. On top of this, early Wednesday morning around 0230, I received my second scolding from Gerry for disturbing his sleep.

(The dogs barking as we were walking between the dormitory and grouper holding ta: and this was keeping Gerry awake.)

Thursday, May 20: We still had 4 males from Wednesday; we had also injected four females. We went to the grouper tank to commence the spawning, but to our amazement we discovered that two of the 4 males had been removed from the tank and the two that were left had no milt. This ended the project. We reported the theft to Gerry and Becky when they arrived to work, but neither seemed to be surprised. Friday, May 21, was a clean up day and we returned home.

Proposed checklist/schedule for the May grouper spawning in Palau: Mark Norman and Jerry Gardner

Sunday, May 16:

GADTC pickup truck with Mark and supplies leaves for airport at 5:00 P.M.

Upon arrival in Palau:

Rent a car from King rental.
Drive to MMDC.
Call Nancy Wang (the Koror state permit has not been issued) to see if she has the permit.
Call Franny Reklai at home and arrange time for fishing on Monday (if you have obtained the Koror permit). If you have not obtained the permit, inform Franny that you must obtain the permit before you can fish in the sanctuary. Therefore, you will not be able to fish early in the morning.

Monday, May 17:

You need to check in with the Director, Becky, and Gerry. Keep these visits brief so you have time to prepare the holding tanks and go fishing.

Check in with Director Idip. Ask him how you can arrange to rent the MMDC boat for a half day later in the week to return any fish that are caught on Monday and Tuesday.
Obtain the original copy of the research fishing permit from his secretary.
Check in with Becky. Ask him which tanks you can use. Let him know that when you spawn the fish, you may need five aquariums (depending on your catch), an electric light (drop light) for working at night, and a simple microscope.
Check in with Gerry Heslinga. Let him know that you will need to purchase styrofoam coolers for shipping the eggs, oxygen for packing the eggs, about 50 small bags, and you may need to use four of his tanks for holding spawners. Ask him about using the freight forwarder to ship the eggs. (Cost, etc.)
Obtain the Koror state fishing permit from John Gibbons (if you don't have it already).

Prepare to go fishing. Supplies needed:

Clipboard, data sheet and pencil
Battery aerators with airlines and airstones
Nets
Lunch, drinks
Copies of the Permits

Catch:

5 females and 6 or 7 males. If the fish bloat, wait until you return to MMDC before bleeding the air bladder.
If possible, make a second trip the same day to catch 5 more females and 6 or 7 more males. If you catch more fish on Monday, you will need to put the females in a separate tank from the first catch.
Otherwise, make arrangements to fish again the next day.
Remember that you need to inject the females before 4:00 P.M. for the Wednesday morning spawning. Therefore, you must return from fishing by 3:30 P.M.

Upon return to MMDC:

Separate males and females into two holding tanks.

Bleed the air bladders of any bloated fish by piercing their abdomen with an injection needle. Do not squeeze the abdomen to force the air out. Allow the fish to swim around with the needle in its body for a couple of hours (or until the bladder has returned to normal).
Later (before 4 P.M.): Weigh the female and inject appropriate amount. Formula: Add 10 ml sterile saline to HCG vial. Inject female with (0.7 X the weight of the fish in kilograms) ml HCG.
Place female in a new tank. Continue this procedure until all females have been injected.
Check flight schedule and/or contact the freight forwarder.
Start gathering the supplies you will need for the spawning and shipping: five aquariums, electric light, four new coolers, etc. Reserve the MMDC boat for returning the spent spawners to the sanctuary on Friday.

Tuesday, May 18:

You will be going fishing again today if you didn't catch enough fish on Monday. Follow the same procedures for holding the fish except you will need four new tanks to keep these fish separate. You may have to use Gerry's tanks for this purpose.
Let the fish acclimate for an hour or so while bleeding the air from any that are bloated.
Give Tuesday's catch or Monday's second catch their first injections.
Give Monday's first catch their second injections 24-hours after their first injections.
Try to get the Certificate of Origin from the Department of Agriculture on Tuesday or else you will have to get it on the way to the airport Wednesday morning.
Get some sleep because you will be up very early to spawn the fish.

Tuesday night:

Keep a record of the hours you work at night. This is the time you will be able to take off when you return to Guam.
Please note that all sea water used in fertilizing, washing and packing the eggs should be filtered through a 5 micron filter bag.
Please read through Appendix 1 of the 1991 report and follow the instructions given in that report if they differ greatly from the instructions given here.
Some exceptions are that in the report they say to wait five hours before skimming the eggs, but obviously you cannot wait that long. Ignore that part and skim the eggs when they are ready. Another point is that the 1991 report recommends shipping at 15,000 eggs per liter. Please use the densities outlined in this report instead.
Cannulate the females nine to ten hours after the second injection to check the egg condition.
Mark should use his best judgement to determine when the eggs are ready.
You must start stripping the females by 4:00 A.M. in order to have the eggs packed in time to make it to the airport.
When the eggs are ready, strip them from the female into a plastic bucket.
Return the female to a new tank.
Use a syringe to collect at least one ml milt from a male. Return the male to the new tank.
Add 100 ml seawater to the bucket with the eggs. Immediately squirt in the milt and gently mix for two to three minutes. This may not work well if you are using a large bucket.

Alternate procedure: Transfer the eggs to a glass beaker and add 100 ml water to the glass beaker.
Immediately squirt in the milt and gently mix for two to three minutes. Don't let the eggs pack into a mass.
Wash the eggs as described in Appendix 1:
Place eggs in a two to four liter container to allow the eggs to float to the top.
Strip eggs from the next female and fertilize as before. Place fertilized eggs in a two to four liter container for washing.
Repeat for the other three fish. Remember to keep the eggs from each fish separate.
Wash the eggs from the first fish by gently pouring or pipetting the floating eggs into another collecting beaker and discarding everything on the bottom.
Repeat for each group of eggs.
Wash each group of eggs a second time.
After the second washing, transfer the eggs into a ten gallon aquarium (if available) for separation of the good eggs for shipping.
Determine the % fertilization of the eggs using the procedure described in Appendix 1 (if you have time).
Increase the salinity of the water to 38 or 40 ppt (if necessary) to get the eggs to float.
Scoop off the floating eggs and place in a clean aquarium (or bucket) until time to pack. Aerate gently.
Preserve a 2-ml or 3-ml sample from each spawn in a labeled vial to bring back to Guam for a count. (If there are enough extra eggs).
Start bagging the eggs at around 6:00 A.M. if possible or as soon as Gerry opens his work area. On Wednesday, you will be packing six coolers with eggs for direct stocking into the GADTC larval tanks and a seventh cooler for the egg shipping density vs. survival trial.
Prepare the six coolers for direct tank stocking by doubling the bags you brought from GADTC and place three doubled bags into each cooler. Add six liters of filtered sea water to each bag. Measure the water very carefully; if anything, add a little bit less than six liters or you will exceed the 45 pound weight limit and Continental will not accept the cooler for shipment. Bubble oxygen into the water in each bag before adding the eggs.
For the six coolers with eggs for direct stocking into the larval culture tank, skim the eggs from the surface of the tank and pour into a 500 ml or one liter cylinder. Measure the volume of eggs floating in the cylinder. Record the volume. There are about 1,000 eggs per ml. Add 18 ml of eggs (18,000 eggs per 6 liters in a bag = 3,000 eggs per liter) from the cylinder to each bag in the cooler. Do this by pipetting the eggs from the cylinder into the bag.
Label each bag in the cooler with the female ID number and number of eggs.
Fill the bags with oxygen and seal with rubber bands as we practiced.
Remember to set aside some eggs from each spawn to determine their hatch rate.
Try to make two coolers each from three different females = 108,000 eggs = 18,000 eggs per bag X 6 bags per cooler. These six bags will be stocked into a 3,000 liter culture tank.
If this works, only the eggs from three females giving the most eggs will be needed.
For the shipping density trial, eggs from a single female will be packed into small bags (twelve small bags in all) in a single cooler. Use cardboard frame (provided by Gerry) in the cooler to support the bags. Double the bags.
Label the bags: four bags will have 1,500 eggs each (1,000 eggs per liter);

four bags will have 7,500 eggs each (5,000 eggs per liter); four bags will have 15,000 eggs each (10,000 eggs per liter).
Add 1.5 liters water to each bag (again, measure the water very carefully to make sure you won't exceed the weight limit.) Bubble oxygen into the water. Add the appropriate volume of eggs to each bag as labelled (1.5 ml = 1,500 eggs; 7.5 ml = 7,500 eggs; 15 ml = 15,000 eggs). Fill the bags with air and seal. Remember that with the small bags there are two layers in the cooler, so leave room for the second layer.
If there is a scale handy (I think Gerry has one in the packing area), weigh the boxes to make sure they are under 45 pounds (20.45 kilograms) each. Seal them with tape and take them to the airport or to the freight forwarder depending on what arrangements you have made.
Obtain the Certificate of Origin on the way to the airport if you did not get it the day before. This must be included with the shipment or the eggs will be confiscated upon arrival in Guam. If you have to declare how many eggs you are shipping, put 400,000 or a lesser number (our imports only allow us to bring in up to 400,000 eggs per shipment).
After delivering the eggs and permits to the airport or freight forwarders, return to MMDC and using the leftover eggs, count the number of eggs in a milliliter. Repeat several times and take an average. This will allow you to calculate the number of ml's to put in each bag for the next morning's shipment (the number may be different from the 1000 eggs per ml you used to estimate for the first shipment).

Wednesday afternoon

Twenty-four hours after the first injection of the Tuesday catch (or the second batch of fish from Monday), give the females their second injection. Follow the previously described procedure.

Wednesday night (Thursday morning)

If possible, phone Mike at home, 734-4232, at around 11:30 P.M. Palau time to find if the eggs hatched OK. This will determine if you need to send extra boxes on Thursday.
Spawn the fish as done the previous night.
Pack at least two coolers from a single spawner for direct stocking (three bags per cooler with 18,000 eggs = 18 mls of eggs per bag). If possible, pack another cooler for the egg density trial.
Repeat the procedures used the previous morning. Again, obtain a Certificate of Origin for this shipment if you don't already have one.
After delivering the eggs, get some sleep.

Friday, May 21:

Return the spent spawners to the sanctuary with the MMDC boat. Pay any outstanding bills at MMDC and prepare to return to Guam.

APPENDIX III

COLLECTION III - JULY 1993

FISHING METHOD: Handlines (3); sardines, squid and reef fish for bait

SPECIES CAUGHT: *Plectropomus areolatus* (5 total), *Epinephelus fuscoguttatus* (11 total - 9 female, 2 male), *E. microdon* (33 total, 5 females, 28 males),

CONDITION OF FISH: *P. areolatus* males were not ripe. *E. microdon* males were ripe. *E. fuscoguttatus* female were ripe.

A total of nine female and male *E. fuscoguttatus* were caught and examined on July 13, 1993. Males of *E. fuscoguttatus* species were difficult to obtain from the wild and only two individuals were caught during the project.

Five *P. areolatus* were caught and examined for their state of maturity. Table below. Previous data regarding the spawning season for this species and the results of the gonadal biopsies indicate that the peak in the spawning season had already passed. After the fish were examined they were returned to the spawning channel.

Table. *P. areolatus* state of maturity

Weight	Sex	State of Maturity
2.42	Unknown	-
2.78	M	Cann sperm
1.66	Unknown	-
2.52	M	Cann sperm
1.42	F	PV

SPAWNING RESULTS: *E. microdon* females possessing vitellogenic oocytes were injected on July 13, 1993 with a priming injection of 2500 IU HCG/kg body weight between 16:30 and 17:30 hours and placed into a 4000 l fiberglass tank equipped with running seawater and continuous aeration. Individual females were marked with various cuts to the caudal fin for identification. Five males were chosen at random, administered a single injection of HCG at a dosage of 1000 IU/kg body weight and placed with the females. Ovarian biopsies were repeated on all females 24 hours after the first (priming) injection and oocyte samples preserved. Also at that time, the females were administered a second (resolving) injection of HCG at a dosage of 700 IU/kg and allowed to spawn naturally.

E. microdon spawning trials

Fish	Weight (KG)	Oocyte Diameter (um)	First Injection IU/Kg	Second Injection IU/kg	# Eggs Spawned x 10^5	Fert. %	Hatch %
TCBW	.79	414	2500	700	8	99.9	95
BC	.87	427	2500	700	8	99.9	95
BW	1.03	454	2500	700	8	99.9	95
BC	1.11	427	2500	700	8	99.9	95
BCBW	1.21	420	2500	700	8	99.9	95

Fertilized eggs from one female *E. microdon* were detected to be undergoing first cleavage at 02:30 on July 15, 1993. The water temperature and salinity in the spawning tank were 27 °C and 34 ppt, respectively. Over the course of the nest two to three hours, the other females were observed spawning. The total number of spawned eggs and the percentage of fertilized eggs were determined from the one

SPAWNING GROUPERS IN PALAU
III

July 12 thru July 18, 1993

GENERAL INFORMATION

PROJECT OBJECTIVES

- To spawn selected species of groupers in Palau and Guam.
- To ship viable grouper eggs from Palau to Guam for larval culture.
- To conduct preliminary larval culture procedures adapting proven methods for a selected species from Palau and Guam.
- To apply developed larval culture procedures to potential aquarium species.
- To establish preliminary guidelines for developing grouper spawning and larval culture in the region for an indigenous species.

PROJECT FUNDING

Pacific Aquaculture Association, U.S. Department of Interior

PROJECT DURATION

PAA - June 1992 thru December 1993

PARTICIPANTS

William J. FitzGerald (EDP, Guam Department of Commerce)
Clyde Tamaru (C's Consultants, Hawaii)
Christine Tamaru (C's Consultants, Hawaii)
Mike Bauerlein (EDP, Guam Department of Commerce, GADTC)

MOON PHASES

Full Moon - July 4, 1993
Last Quarter Moon - July 12th
New Moon - July 19

FIRST DAY'S CATCH

DATE:	July 13, 1993
LOCATION: of seaward jutting reef	Ngerumekaol (Ulong) Channel: outer third of channel to the southern point
TIDES:	Low tide 2.1 ft at 0812, High tide 4.2 ft at 1353,
MOON PHASE:	One day after last Quarter Moon
FISHING TIME:	1030-1430 (4 hours);
FISHERMEN:	Thomas Taro
TRAVEL TIME:	45 minutes each way

spawning tank. The fecundity of individual fish was estimated by dividing the total number of spawned eggs by the number of females (five). The percentage of fertilized eggs was found to be very high (i.e., 99.9%) and the hatch rate from this group spawning was also observed to be very high (i.e., > 95%).

E. fuscoguttatus were treated as described for *E. microdon* with the exception of the dosage of HCG administered. An ovarian biopsy performed on female BC at approximately 04:30 on July 15, 1993, found her to have ovulated completely. She was then strip spawned and eggs were manually fertilized at 05:05 using the dry method. The resulting fecundity, fertilization, and percent hatch of the spawned eggs are presented in Table below. Females identified as TC, TW, BW, and TCBC were allowed to complete final maturation in the same tank. However, they did not spawn and the ovulated eggs were manually stripped on the afternoon of July 16, 1993, as the ovulated oocytes by this time period were overripe and nonviable.

Both *E. fuscoguttatus* males were administered HCG as described for *E. microdon* and were found to respond in similar fashion. In addition, a single male *E. fuscoguttatus* held captive at MMDC for public exhibit was examined for milt. The gonad biopsy, however, was negative.

SHIPPING OF EGGS: Fertilized eggs of *E. microdon* and *E. fuscoguttatus* were packed at a density of 5,000 eggs/l and air-transported to GADTC to conduct larval rearing trials.

SECOND DAY'S CATCH

DATE:	July 14, 1993
LOCATION:	West Channel (7 • 16.9'N 134 • 15.2'E)
TIDES:	Low tide 1.9 ft at 0924, High tide 4.1 ft at 1517,
MOON PHASE:	Two days after Last Quarter Moon
FISHING TIME:	900-1300 (4 hours)
FISHERMEN:	Thomas Taro
TRAVEL TIME:	45 minutes each way
FISHING METHOD:	Handlines (2); sardines & tuna for bait
SPECIES CAUGHT:	*E. microdon* (2 females)

CONDITION OF FISH: On July 14, 1993, an additional two *E. microdon* females were caught and placed with the same group of males that participated in the first group spawning.

SPAWNING: Their hormone treatment was initiated on that same day between 16:30 and 17:30 at a dosage of 700 IU/kg followed 24 hours later by a resolving injection of 1400 IU/kg. Spawning was detected for TCBC on July 16, 1993, approximately 12 hours after the resolving injection. Although TC underwent hydration, she did not spawn.

E. microdon spawning trials

Fish	Weight (KG)	Oocyte Diameter (um)	First Injection IU/Kg	Second Injection IU/kg	# Eggs Spawned x 10^6	Fert. %	Hatch %
TCBC	1.23	415	700	1400	1.6	32.6	0
TC	1.29	369	700	1400	NO SPAWN	-	-

THIRD DAY'S CATCH

DATE: July 17, 1993

LOCATION: Patch reefs inside barrier reef Rock Islands 7 Beaches area (7 • 16.9'N 134 • 15.2'E)

TIDES: Low tide 0.6 ft at 1218, High tide 4.9 ft at 1842,

MOON PHASE: 5 days past Last Quarter Moon

FISHING TIME: 0930-1230 (3 hours);

FISHERMEN: Thomas Taro

TRAVEL TIME: 1 hr 15 minutes (45 minutes to release fish in Ulong Channel)

FISHING METHOD: Handlines (2); sardines & tuna for bait

SPECIES CAUGHT: *Cephalopholis miniata* (5 live undetermined sex, 3 dead), *Cephalopholis urodeta* (1 undetermined sex), *Variola albimarginata* (1 undetermined sex), *Variola louti* (3 undetermined sex), *Plectropomus leopardus* (1 undetermined sex), *Plectropomus areolatus* (2 undetermined sex), *Epinephelus fasciatus* (1 undetermined sex).

CONDITION OF FISH: Two *Aethaloperca rogata* reported by the fisherman to have eggs. This species is reported to spawn year round. The three dead *C. miniata* were dissected and examined as to sex and state of maturity. One was in transition from female to male. The other two were one female and one male.

Table. *C. miniata* sex and state of maturity

Date	Total Length	Weight	Sex	Maturity
7/17/93	20.5	183	F	PV + Vit
7/17/93	26.2	452	F/M	Transitional
7/17/93	28.5	516	M	Cann sperm

SPECIES INFORMATION:

A. *Plectropomus areolatus* (was *P. truncatus*)

1. Starts aggregating/spawning March thru June
2. Preferred species for food. Stated to have a higher fat content and tenderer flesh.
3. Most common species of *Plectropomus* sps. in reef fronts and channels in Palau.

4. In spawning season catches of *P. areolatus* consisted of 60% to 85% of the total number of fish caught.

B. *Plectropomus leopardus*
   1. Starts aggregating/spawning March thru June
   2. Preferred species for food. Stated to have a higher fat content and tenderer flesh.
   3. Potentially of higher market value than *P. areolatus* in the Japanese market, since the fish has a bright red coloration when chilled (*P. areolatus* has a grey-green color with black spots).

C. *Epinephelus microdon* (was *E. microdon*)
   1. Aggregates/spawns May thru August, but mainly June/July
   2. Flesh can be tough.

D. *E. fuscoguttatus*
   1. Aggregates/spawns May thru August, but mainly June/July
   2. Preferred of the *Epinephelus* sps, mainly because of its bigger size.

APPENDIX IV

COLLECTION IV - AUGUST 1993

SPAWNING GROUPERS IN PALAU
IV

August 12 thru August 18, 1993

GENERAL INFORMATION

PROJECT OBJECTIVES

- To spawn selected species of groupers in Palau and Guam.
- To ship viable grouper eggs from Palau to Guam for larval culture.
- To conduct preliminary larval culture procedures adapting proven methods for a selected species from Palau and Guam.
- To apply developed larval culture procedures to potential aquarium species.
- To establish preliminary guidelines for developing grouper spawning and larval culture in the region for an indigenous species.

PROJECT FUNDING

Pacific Aquaculture Association, U.S. Department of Interior

PROJECT DURATION

PAA - June 1992 thru December 1993

PARTICIPANTS

William J. FitzGerald (EDP, Guam Department of Commerce)
Clyde Tamaru (C's Consultants, Hawaii)
Christine Calstrom-Trick (C's Consultants, Hawaii)
Mark Norman (EDP, Guam Department of Commerce, GADTC)

MOON PHASES

Full Moon - August 2, 1993
Last Quarter Moon - August 11th
New Moon - August 18

FIRST DAY'S CATCH

DATE:	August 12, 1993
LOCATION:	West Channel: Ngeremlengui (NW of Ngetpang Bay)
TIDES:	Low tide 2.1 ft at 0828, High tide 4.2 ft at 1430,
MOON PHASE:	1 day after last Quarter Moon
FISHING TIME:	0945-1400 (4 hours);
FISHERMEN:	Thomas Taro
TRAVEL TIME:	45 minutes each way
FISHING METHOD:	Handlines (3); tuna and reef fish for bait

SPECIES CAUGHT: *Epinephelus microdon* (14; 4 females, 10 males - 2 ripe 2 marginal and the rest not ripe). *E. fuscoguttatus* (1 female)

CONDITION OF FISH: Eggs were sampled from all of the females.
None of the fish were injected on the day of catch. Waiting to get a male E. fuscoguttatus to spawn.

Species	Sex	Weight (kg)	Total Length (cm)	
Epinephelus microdon				
1	F	0.64		Died next day
2	F	1.03		
3	F	1.19		
4	F	1.27		
5	M	1.38	42.55	
6	M/F	1.35		
7	M	1.58	47.63	
8	M	1.50		
9	M	1.36		
10	M	1.39	46.36	
11	M	1.47	45.09	
12	M	1.53	46.36	
13	M	1.33	44.45	
14	M	1.59	49.53	
Epinephelus fuscoguttatus				
1	F	4.35		

SECOND DAY'S CATCH

DATE: August 13, 1993

LOCATION: Ulong Channel (7 • 16.9'N 134 • 15.2'E) and Rock Isl. area

TIDES: Low tide 1.8 ft at 0953, High tide 4.3 at 1610,

MOON PHASE: 2 days after Last Quarter Moon

FISHING TIME: 0915 start (2 hours at Ulong and 2 hours on patch reefs in Rock Isl)

FISHERMEN: Thomas Taro

TRAVEL TIME: 45 minutes each way from T-dock

FISHING METHOD: Handlines (2); reef fish, sardines & tuna for bait

SPECIES CAUGHT: At Ulong channel - *E. microdon* (7 were kept 2 females and 5 males; 9 male caught measured and checked for condition of maturation then released). At patch reefs in Rock Isl. - C miniata (6), *P. leopardus* (2), *Variola louti* (2), *Cephalopholis urodeta* (2), *Aethaloperca rogaa* (1), *Cephalopholis sonneratti* and a number of snappers (6) and trigger fish - *Sufflamen chrysoptera* (1).

Species	Sex	Weight (kg)	Total Length (cm)	Condition (cannulated)
Epinephelus microdon				
1	M		*44.45*	Sparse
2	M		47.63	Sparse
3	M		45.09	Sparse
4	M		*49.53*	Sparse
5	M/F		35.56	Negative
6	M		45.72	Sparse
7	M		*46.36*	Sparse
8	M		48.26	Can. sperm
9	M		48.26	Running
10	M	*2.05*	*50.17*	Can. sperm
11	M	1.46	46.06	Can. sperm
12	M	1.45	45.72	Can. sperm
13	M	*1.24*	*44.45*	Can. sperm
14	M	1.51	48.26	Can. sperm
15	F	1.34	43.82	Vit
16	*F*	*1.33*	*43.82*	*Vit*
Cephalopholis miniata				
1	F	.37	27.94	
2	M	.54	33.97	Can
3	Unk	.49	32.07	neg
4	Unk	.47	30.48	neg
5	Unk	.32	30.48	neg
6	M	.33	28.58	?
Variola louti				
1				
2				
Cephalopholis urodeta				
1				
2				
Aethaloperca rogaa				
1	Unk	.99	40.96	Neg
Plectropomus areolatus				
1	Unk	.50	33.02	
2	Unk	.50	35.24	
Cephalopholis sonneratti				
1	Unk	.52	31.75	

CONDITION OF FISH:

Injection of fish from 1st and 2nd day fishing

Species	Sex	Weight (kg)	1st Injection - 1700 (8/14/93)	2nd injection - 1600 (8/15/93)
Epinephelus microdon				
TC	*F*		*.15*	*.30*
TW	*F*		*.17*	*.34*
BW	*F*		*.26*	*.52*
	M	*2.05*	*.1*	
	M	*1.46*	*.1*	
	M	*1.45*	*.1*	
	M	*1.24*	*.1*	
	M	*1.51*	*.1*	

Priming injection for females dose was 700 IU/kg HCG
Resolving injection for females dose was 1400 IU/kg HCG
Males injected at approximately 500 IU/kg HCG (single injection)

SPAWNING RESULTS: All three female ı spawned. The induced spawns occurred 5 to 12 hours after the resolving injection at approximately _ 30 (BW), 0230 (TW) and 0500 (TC). The spawns from BW and TW were 98.5% and 98.3% fertilized respectively. The TC had a fertilization rate of 70.1%. The fecundity was 1.16 x 10^6 eggs/fish for BW and TW. The fecundity for TC was 1.13 x 10^6 eggs/fish.

SHIPPING: The shipping density was as presented in the table below. Egg density in the incubation tank was 5,545 eggs/l. Seven liters of 40 PPT seawater was the water volume in each large shipping bag. Two large bags per styrofoam cooler. One liter volume in small bags with 10 bags per cooler. Packing time was 1 hr 30 min. The total time in the bag from time of packing to being placed in the designated tank at GADTC was 9 hrs.

Targeted GADTC tank	# of Eggs Shipped	Density in bag (eggs/l)	Tank Volume (liters)	Larvae Density in tank** (larvae/l)
LRT 1	70,000	10,000	1,000*	35
LRT 2	70,000	10,000	1,000*	35
LRT 3	70,000	10,000	1,000*	35
LRT 4	70,000	10,000	1,000*	35
Extensive tank	280,000	20,000	15,000	9.3
S-7 Extensive tank	420,000	20,000	150,000	1.9
Experimental tanks (15 tanks @ 1,000/tank))	15,000	1,000	15	33.3
Total	995,000			

*Initial volume to allow for proper density of oyster trochophores in first feeding. Total working volume is 3,000 l.
**Based on estimated 50% hatch.

HATCHING: The hatch rate measured at MMDC was 91.4% for the eggs shipped to Guam. The hatc.. rate measured at GADTC was > 90%.

SHIPPING OF FISH: Two live *E. microdon* (males) were shipped as check in baggage with the return (one of the GADTC staff. Fish were cooled to 24 C prior to packing. They were placed one fish per

cooler in 13.5 l of seawater at 24 C. Doubled bagged. A bag of blue ice was added to each box. The fish on arrival were in excellent condition. The water temperature on arrival was 22 C. The fish were active and were feeding the following day when food was offered.

THIRD DAY'S CATCH

DATE: August 16, 1993

LOCATION: Inside barrier reef from just north of Ngetpang Bay to south on patch reefs inside lagoon: Mesikm, Miich, Edldukl

TIDES: Low tide 0.5 ft at 1239, High tide 5.6 ft at 1857

MOON PHASE: 5 days past Last Quarter Moon

FISHING TIME: 1000-1500 (5 hours);

FISHERMEN: Thomas Taro

TRAVEL TIME: (45 minutes)

FISHING METHOD: Handlines (4); sardines, squid & tuna for bait

SPECIES CAUGHT: The target species was *C. miniata*. 47 of *C. miniata* were caught (3 died coming in, two additional fish died prior to departure). Other species caught included *E. merra* (4), *Plectropomus leopardus* (8), *C. spiloparaea* (6), *E. macrospilos*, and numerous other reef fish (totalling approximately 150 lbs).

CONDITION OF FISH: The fish were not checked, since they were week from the trip in and they were intended for broodstock in Guam. However, the 3 fish that died on the trip in from fishing were examined and all three were females. One indicated recent (within a couple days) ovulation and another indicated near spawning. The third fish was an immature female. The two additional fish that died while being held in the tank were not checked. Information on these samples are presented in the table below.

Fish	Total Length (cm)	Weight (g)	Sex	Condition
1	21.5	122	F	Ripe
2	23	175	F	Recent spawn
3	23	151	F	Not mature
4				
5				

SHIPPING FISH: Sixteen *C. miniata* were shipped by air cargo on 8/17/93. Five larger fish were sent 1 fish/box, four boxes with medium size to small fish were sent 2 fish/box, and one box with three fish were sent 3 fish/box. The shipping bags consisted of two Continental Airlines bags filled with 12 l of water cooled to 23° C. The shipping time was approximately 6 hrs. All fish survived. All of the inner bags had leaked water; however, sufficient water remained in the inner bag to keep the gills wet. All of the fish will undergo a two week treatment of antibiotic baths.

An additional 28 *C. miniata* were shipped to GADTC on 8/18/93. Packing temperature was lowered to 20° C. The fish were acclimated from 28° C to 23° C over a one hour period prior to packing. All of the fish survived the 9 hr transport time.

Five *E. microdon* were shipped as check in baggage on 8/17/93. Three larger fish were shipped 1 fish/box. Two smaller fish were shipped 2 fish/box. All fish survived in good condition. The shipping bags consisted of two Continental Airlines bags filled with 12 l of water cooled to 23° C. The shipment time was approximately 4 hours. The water was cooled to 24° C for shipment. Blue ice was placed inside the cooler at one end to maintain the reduced temperature.

Two *E. microdon* juveniles (estimated to be 2-3 months old) and four juvenile *E. merra* were shipped as part of the above checked in baggage. All six fish were placed in one bag with 4 l of water. All fish survived the 4 hour shipment. The water temperature was not lowered prior to packing.

APPENDIX V

SHIPPING LIVE ADULT EPINEPHELUS MICRODON TRIAL

SHIPPING LIVE ADULT EPINEPHELUS MICRODON TRIAL

PURPOSE:

To determine if adult groupers could be transported to Guam to establish a broodstock.

METHOD:

Two adult E. microdon were used as the test animals. They were placed in a 30 gallon aquarium filled approximately half-way. The initial temperature was taken then the water temperature was lowered to the target transport temperature using ice floated in sealed plastic bags. When the target transport temperature was reached, the fish were transferred to large plastic bags (3 mil thickness, Continental Airlines plastic bags used to cover ice coolers). The shipping bags were double bagged. The inner bag was filled with 12 liters of water (lowered to the target temperature). One fish, which was acclimated at the lowered the shipping temperature, was placed in each shipment container. Oxygen was added and the bags were sealed. Two initial shipment temperatures were used (25 and 22° C). Ice was added to the cooler to help maintain the shipping temperature. The condition of the fish were examined every two hours after the initial four hours of the trial to determine if they were still alive.

The lowering of the water temperature was done over a 45 minute period from the ambient 28.5 to 25° C. An additional 10 minutes was taken to bring the temperature to 22 degrees.

RESULTS:

Both fish survived the trial shipping. The fish were transferred to a holding tank at the termination of the trial. Both fish recovered immediately and were able to maintain their equilibrium and swim; even though, their reaction time was depressed initially.

Summary of Transport Trial

Time	Condition of Fish	
	25° C	22° C
1600 Initiation	live	live
2000	live	live
2200	live	live
2400	live	live
0200*	live	live
0400 Termination	live	live
Initial Water Temperature	28.5	28.5
Final water Temperature	19	23.5
Weight of fish	0.91 kg	0.66 kg

*The inner plastic bag of the 22 degree C trial was punctured and bag was partially deflated. Outer bag maintained its integrity.

The temperature in the 25° C target transport decreased during the trial to 19° C. This was due to excess ice placed in the cooler chest to maintain the lowered temperature.

CONCLUSION:

The fish tolerated the lower water temperature which slowed down their movement and metabolism and reduced the stress of transportation and handling. The fish that was initially at 25° C, but dropped to 19° C during the trial period, was noticeably slower moving during initial recovery and during the periodic checks the gill operculum movement was slower than the other fish. Further trials are needed to determine what would be the optimum water temperature to maximize the transport time while maintaining the fish in good condition. Additional trials are needed to optimize the shipping system while reducing the total cost of shipping (weight). Trials on other species of interest for cultivation should be conducted to facilitate the establishment of a broodstock in Guam. This would reduce future costs involved in working with these species (travel to Palau and associated collection and operation costs in Palau). It would also allow for additional work to be conducted that is not carried out in Palau due to the requirements of meeting flight shipment schedules and time constraints.

APPENDIX VI

SHIPMENT OF ADULT *EPINEPHELUS FUSCOGUTTATUS* TO GUAM

SHIPMENT OF ADULT EPINEPHELUS FUSCOGUTTATUS TO GUAM

PURPOSE:

To establish broodstock of Epinephelus fuscoguttatus at Guam Aquaculture Development and Training Center.

METHOD:

Three female E. fuscoguttatus were selected based on size. Smaller fish were selected from the catch made on July 14, 1993 from the West Channel in Palau. The fish ranged in size from 2 - 3 kg.

The fish were prepared for shipment by lowering the water temperature from the ambient temperature of 28° C to 23° C over a one hour period. Ice sealed in plastic bags and floated in the tank was used to lower the water temperature. The packing method followed the trial shipment of E. microdon.

RESULTS:

Two of the fish survived the shipment. The time from sealing for shipment and the opening of the boxes was approximately 5 hours. The temperature in the shipment water dropped in all boxes below the intended 18-20° C. Temperature dropped between 9 -13° C in the shipping water.

The fish were transferred to a transfer tank upon arrival. The fish were initially not responding and had to be massaged and kept moving to resuscitate them. Approximately 1-hour of resuscitation was required. The fish were very weak. Attempts to feed the fish over the next two days were unsuccessful. The two surviving female fish died 3-days later.

Shipping Summary

	Fish 1	Fish 2	Fish 3
Weight (kgs)	2.69	3.21	3.35
Sex	F	F	F
Initial Ambient Temperature (C)	28	28	28
Initial Packing Temperature (C)	23	23	23
Termination Temperature (C)	9	12	13
Reacclimatization Temperature (C)	27	27	27
Shipping time (hours)	5	5	5
Survival	Y	Y	N

CONCLUSION:

The temperature in the shipping boxes dropped too low. Excess ice was placed in the container. It is recommended that future shipments utilize blue ice placed on the top or side of the container. This would avoid direct contact with the shipping water. Crumpled newspaper or foam packing material could be utilized as insulation.

APPENDIX VII

AQUARIUM SPECIES OF GROUPERS FOR CULTURE

AQUARIUM SPECIES OF GROUPERS FOR CULTURE

PURPOSE:

The identification of appropriate species of groupers that would potentially be suitable for cultivation for the tropical aquarium fish trade.

METHOD:

With the assistance of a private fisherman, approximately 3 hours were spent fishing (not counting time moving between sites) on patch reefs inside the barrier reef on the west side of Palau around the Rock Islands and 7 Beaches area. The fishing method consisted of handlines (3) with mackerel and squid for bait. The fish were transferred live to the Micronesian Mariculture Demonstration Center (MMDC) and held in tanks. Some fish had extended gas bladders and were partially relieved of the pressure with a hyperdermic needle.

The criteria for selecting an appropriate species for the aquarium trade consisted of the following:

- Colorful
- Active swimming behavior or non sedentary or cryptic
- Behavior suitable as pet in aquarium
- Adaptability to living in captivity in a tank
- Broodstock readily available (male and female)
- Larval culture feasible

RESULTS:

Seven species of groupers were collected (July 1993) that offered some potential as aquarium species. The initial collection consisted of the following:

Table 1. Groupers collected

Species	No. July	No. August	Sex
Potential aquarium species of groupers			
Cephalopholis miniata	8	53	3-M, 5-F, 1-T, undetermined
Cephalopholis urodeta	1	2	undetermined
Cephalopholis spiloparaea	1	6	undetermined
Cephalopholis sonnerati		1	undetermined
Epinephelus fasciatus	1		undetermined
Epinephelus merra		4	undetermined
Variola louti	3	2	undetermined
Variola albimarginata	1		undetermined
Non-aquarium species of groupers			
Plectropomus leopardus	1	8	undetermined
Plectropomus aerolatus	2	2	undetermined
Aethaloperca rogaa	2	1	female - ripe
Epinephelus microdon	4	16	11-M, 2-F, 1-T, 4 undetermined

Other species include various snappers, goat fish, and wrasses.

A description of each potential aquarium species after Myers (1989) with additional physical description is provided as follows.

Cephalopholis miniata - Standard length to 31 cm. Common name - Coral Grouper. The coloration of both juveniles and adults consist of large round evenly spaced black outlined blue spots on a red background. It typically inhabits channels and outer reef slopes in areas of rich coral growth and clear water at depths of 3 to 150 m or more. It occurs solitarily or in small groups. It is common on the leeward barrier reefs of Palau and the Carolines, but rare in the Marianas.

It is reported to occur in pairs and to spawn year round with spawning at dusk or dawn (personal communications, Larry Sharon).

Cephalopholis urodeta - Standard length to 19 cm. Common name - Flagtail Grouper. This small grouper occurs on lagoon and seaward reefs at depths of 1 to 36 m. It is particularly common on shallow clearwater seaward reefs at depths of 3 to 15 m. It is generally solitary and feeds on small fishes and crustaceans.

Cephalopholis sonnerati - Standard length to 47 cm. Common name - Tomato grouper. This colorful grouper inhabits deep lagoon reefs and steep outer reef slopes at depths of 12 to 150 m. It is uncommon in less than 30 m, but show up regularly in catches of bottom fish. It feeds on small fishes and crustaceans including shrimps, crabs, and stomatopods. Females mature at 28 cm, and males mature at 34 cm and spawning coincides with the April-May and October rainy seasons in E. Africa. The one specimen caught was from less than 30 m inside the barrier reef. It was a deep maroon crimson color. Approximately 35 cm long.

Cephalopholis spiloparaea - Standard length to 19 cm. Common name Orange-red pigmy grouper. The color of this little grouper may range from a dull orange to a deep maroon or crimson. It is the deep water counter part of C. urodeta, inhabiting steep outer reef slopes at depths of 16 to at least 108 m. It is rare in less than 30 m, but quite common deeper.

Variola louti - Standard length to 56 cm. Common name - Lyretail Grouper. Coloration is different between juvenile, sub-adults and adults. The juvenile and sub-adults generally are very attractive with a purple/maroon background and white spots. The long trailing tail is an attractive characteristic. It inhabits deep lagoon pinnacles, channels , and seaward reefs at depths of 4 to at least 150 m. Adults seem to prefer areas of rich coral growth below 15 m; but juveniles may occur in as little as 4 m. Its diet consists primarily of fishes, including relatively large spiny or venomous species such as squirrel fishes and scorpion fishes, and to a lesser extent on crabs or spiny lobsters. Large individuals may be ciguatoxic in certain areas.

The fish behaved were very skittish in the holding tank. When a net was placed in the tank to catch other fish the Variola would frequently jump out of the tank. The coloration fades to a very washed out color when in a light tank.

Variola albimarginata - Standard length to 45 cm. Common name - Whitemargin Lyretail Grouper. It is relatively rare. It inhabits outer reef slopes and coastal reefs at depths of 12 to 90 m.

Epinephelus fasciatus - Standard length to 29 cm. Common name - Black-tipped Grouper. This is one of the most common groupers of outer reef slopes at depths below 15 m. In protected bays and lagoons, it may occur as shallow as 4 m and has

been fished from as deep as 160 m. It is generally solitary and feeds primarily on crustaceans and fishes, and to a lesser extent on octopods and ophiuroids, by day as well as at night. Off East Africa females of E. fasciatus mature at 16 cm, males at 17.5 cm, and spawning takes place seasonally prior to the major rainy season.

Epinephelus merra - Standard length to 22.5 cm. Common name - Honeycomb Grouper. The honeycomb grouper is a common resident of shallow lagoon and semi-protected seaward reefs that occasionally occurs as deep as 50 m. Juveniles often occur in thickets of staghorn Acropora corals. E. merra feeds primarily on fishes as it grows.

The three C. miniata that died were opened to examine the gonads to determine the sex and if they were mature. The results are presented in Table 2. Examination of the gonads revealed that they had spawned in the last 1-2 months. Sperm could be activated.

Table 2. C. miniata

Specimen	SL (cm)	Weight (g)	Sex	Comment
1	26.3	452	M/F	Transitional female
2	28.5	516	M	Sperm activated with seawater
3	20.5	183	F	PV and few vit eggs

CONCLUSION:

C. miniata is the first choice for cultivation for the aquarium trade. C. urodeta is the second choice. V. louti would have the added advantage of use as a food species, since it occurs in the commercial fisheries market. Of the species collected the two most outstanding species based on appearance were C. miniata and V. louti. The coloration was uniform between the different sizes of C. miniata. V. louti demonstrated color phases that it apparently goes through as it matures. It also faded drastically to a pale color in a light colored tank.

The priority order of the potential aquarium species examined is presented in Table 3. This is a preliminary evaluation of the species considered based on only the first four factors of the criteria for selection as an appropriate aquarium species. The latter two criteria could not be adequately evaluated at this time. However, C. miniata seems to have an adequate readily catchable broodstock and was the dominate species in the catch of aquarium type species. Some of the other species (e.g., V. albimarginata) are considered rare and would be difficult to obtain adequate numbers of adult fish as broodstock.

Table 3. Species priority list for aquarium groupers

Priority	Species
1	Cephalopholis miniata
2	Cephalopholis urodeta
3	Cephalopholis spiloparacea
4	Variola louti
5	Variola albimarginata
6	Cephalopholis sonnerati
7	Epinephelus fasciatus
8	Epinephelus merra

Based on desirable behavior and "friendliness" the C. miniata was the superior species. Wild caught species showed signs of curiosity when an observer looked in the tank. The fish would come to the surface and look at the person. C. miniata held in captivity at MMDC for approximately three months would come to the surface when being fed and take the food from the attendant's hand. Other species showed degrees of behavior from sitting on the bottom and not moving to that described for C. miniata. C. urodeta demonstrated behavioral characteristics that also make it a preferred aquarium species. However, V. louti demonstrated a nervousness in the tank and frequently jumped out of the tank when a net was placed in the tank.

A second effort to collect aquarium species the following month (August 1993) was made for the selected priority species of C. miniata. The catch consisted of 53 C. miniata. Of these, 44 were sent to Guam to establish an initial broodstock for this species.

APPENDIX VIII

OBSERVED SPAWNING BEHAVIOR OF EPINEPHELUS MICRODON IN CAPTIVITY

OBSERVED SPAWNING BEHAVIOR OF EPINEPHELUS MICRODON IN CAPTIVITY

The spawning behavior of E. microdon was observed during two separate spawnings of groups of E. microdon held in a tank at Micronesian Mariculture Demonstration Center. The spawnings were induced through injections of HCG to mature females (2 injections) and males (1 injection). The chronology of the spawning behavior was recorded in the second group of fish spawned and is presented in Table 1.

Table 1. Chronology of Spawning and Spawning in Epinephelus microdon

Activity	Time	Day
Priming Injection	1800	7/13
Resolving Injection	1800	7/14
Initial Movement of Males	0100-0200	7/15
Initial Movement of Female	0400	7/15
Onset of tail waging and head shaking	0430	7/15
Increased	0530	7/15
Initial upward swimming	0545	7/15
Spawning	0640	7/15

The first group consisted of 5 females. All females spawned naturally in the tank approximately 12 hours following the resolving injection. These females were injected with a high priming injection of HCG (2,500 IU/kg) and a lower resolving injection (700 IU/kg).

The second group consisted of 2 females and 7 males (same 5 males used in the previous trial plus 2 additional males). Only one of the females spawned naturally. The second fish responded to the HCG injections (i.e., ovulation), but it did not result in a natural spawn. Both females received a priming injection of 700 IU/kg followed 24 hours later with a resolving injection of 1400 IU/kg. The second female did not exhibit the spawning behavior nor did it elicit the spawning behavior by the males. The female that did not spawn nor demonstrate the spawning behavior possessed the smallest egg diameter that could possibly indicate a critical oocyte diameter of >400 um for this species. Three female E. fuscoguttatus that were injected with 700 IU/kg for the priming injections and 1400 IU/kg resolving injection as well as two that were injected at 250 IU/kg and 500 IU/kg for the priming and resolving injections respectfully did not display spawning behavior nor did natural spawning occur. One control female was not injected with HCG and did not ovulate or display spawning behavior. The ovulated fish had to be stripped spawned. Fish that were not stripped spawned near ovulation had to be forcibly stripped (approximately 48 hrs later) to eliminate the degenerated eggs.

Prior to the onset of spawning behavior the fish laid inactive along the bottom side of the tank. They were often 2 - 4 fish deep or bunched together. The male fish showed the first activity by moving away from the side and bottom of the tank and started to slowly swim about the tank. This was followed later by the female moving away from the bottom side; even though the female was upright, swimming activity was still limited to small movements around the bottom area of

the tank. The next significant behavior change was the onset of tail waging as the males swam slowly past the female. Similarly, head shaking was initiated as the males swam closely past the female. Increased territorial behavior by the males against other males was also observed at this time. The frequency of tail waging and head shaking increased over the next two hours prior to actual spawning. The culminating behavior was an upward swimming to the surface by the female which was followed by 2 to 4 males. The males swam close to the female usually in physical contact. A swirling swimming behavior was also observed at the surface. This upward swimming behavior by the female occurred three times with actual spawning with the complete release of the eggs on the third upward swim. Between each of the upward swimming behaviors the female would settled back to the bottom of the tank. Males would continue the tail waging and head shaking behavior during this interlude as well as the territorial behavior.

APPENDIX IX

GADTC TECHNICAL REPORT NO. 16: PRELIMINARY SPAWNING AND LARVAL CULTURE TRIALS FOR GROUPERS FROM GUAM AND PALAU

Preliminary Spawning and Larval Culture Trials for Groupers from Guam and Palau

TECHNICAL REPORT NO. 016

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GUAM AQUACULTURE DEVELOPMENT AND TRAINING CENTER

DEPARTMENT OF COMMERCE
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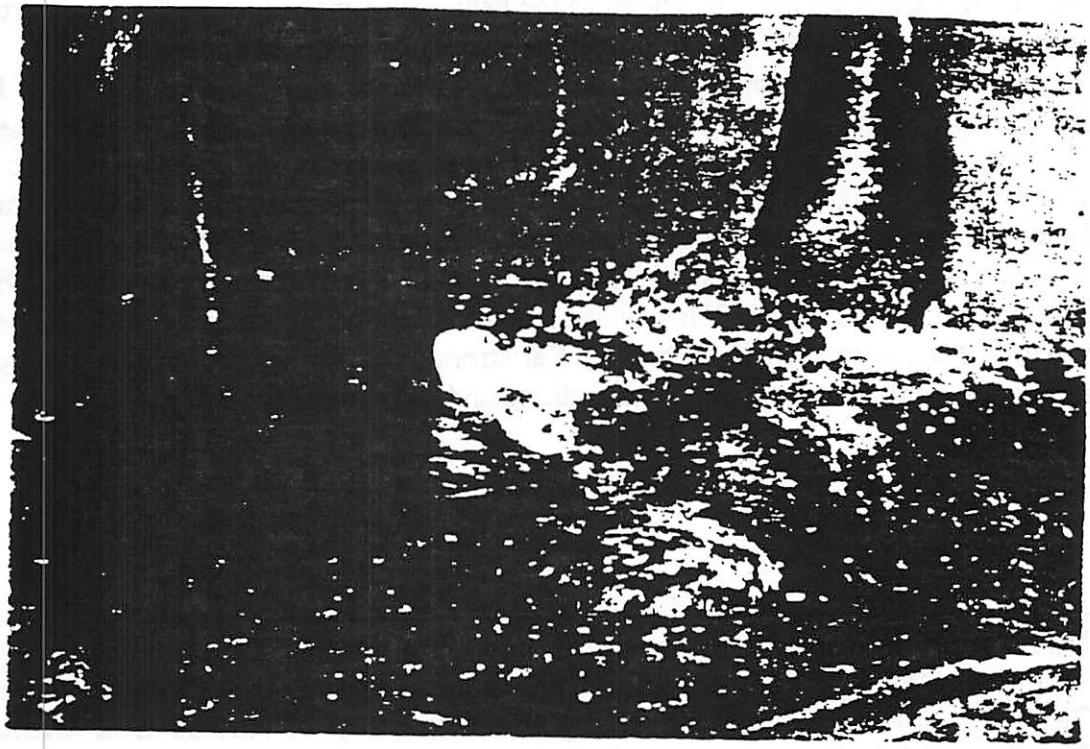
Preliminary Spawning and Larval Culture Trials For Groupers from Guam and Palau

Final Report
July 2 - 24 and August 6 - 24, 1993



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Cover photograph by William J. FitzGerald, Jr.

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EXECUTIVE SUMMARY

This report summarizes the activities of the short-term consultancy focused on the development of hatchery technology for the artificial propagation of groupers in Guam and Palau. The two phases of the consultancy were July 2-24 and August 6-24, 1993, and were accomplished at the Micronesian Mariculture Demonstration Center (MMDC) in Palau and at the Guam Aquaculture Development and Training Center (GADTC) in Guam. Accomplishments during this period were:

1. Defined the state of maturity of female *E. microdon* necessary to initiate hormone treatment for induction of final maturation and spawning. Characterized the *in vivo* changes in oocyte diameters that accompany hormonal induction of final maturation and spawning.

2. Production of fertilized eggs (7×10^6) and larvae from *Epinephelus microdon* by hormone (i.e., HCG) induced spawns.

3. Defined the state of maturity of female *E. fuscoguttatus* necessary to initiate hormone treatment for induction of final maturation and spawning. Characterized the *in vivo* changes in oocyte diameters that accompany hormonal induction of final maturation and spawning.

4. Production of fertilized eggs (1.5×10^6) and larvae from *E. fuscoguttatus* by a hormone induced spawn.

5. Characterized length x weight relationship, size frequency distribution, sex composition by size, size at sexual transition from female to male for *E. microdon* and *Cephalopholis miniata*.

6. Defined the salinity for the activation of sperm from *E. microdon* and *E. fuscoguttatus*.

7. Tested various solutions for use as sperm extenders using sperm from *E. microdon*.

8. Characterized the size frequency distribution of recently spawned and mature *C. miniata* females to determine the critical oocyte size for the induction of spawning.

9. Characterized embryonic development for *E. microdon* and *E. fuscoguttatus*.

10. Defined the optimum egg density for transporting fertilized grouper eggs. Successfully transported 700 x 10^3 fertilized eggs from MMDC in Palau to GADTC in Guam.

11. Completed experiments for the transport of adult *E. microdon* and *E. fuscoguttatus*. Successfully transported six adult *E. microdon* and 46 adult *Cephalopholis miniata* broodstock from MMDC in Palau to GADTC in Guam.

12. Characterized the fatty acid profiles of spawned eggs from *E. microdon* and *E. fuscoguttatus*.

13. Reviewed and improved the production of rotifers at GADTC. Improved the nutritional (i.e., fatty acid profile) quality of rotifers at GADTC. Established contact to obtain a smaller size rotifer (SS-type) for mass culture and use for larval-rearing of grouper.

14. Established the methodology for obtaining copepods from shrimp broodstock raceways at GADTC for use in grouper larval-rearing trials. Characterized the relative abundance of copepods in relation to standard raceway maintenance procedures for shrimp broodstock.

15. Completed small-scale (i.e., 15-liter), intensive (i.e., 3000-liter) and extensive (20,000 - 100,000-liter) larval rearing experiments and rearing trials for *E. microdon* and *E. fuscoguttatus*.

16. Production of 15-day-old posthatched *E. fuscoguttatus* larvae.

17. Determined the effects of light on newly hatched *E. microdon* larvae through first feeding.

18. Determined the survival rate of rotifers, copepods, medusae and *Artemia* nauplii under acute formalin treatments.

19. Collected and submitted oyster trochophores, newly hatched and enriched *Artemia* nauplii, *Penaeus stylirostris* nauplii, copepods, and enriched rotifers for fatty acid analysis.

20. Located source of the sea urchin, *Tripneustes gratilla*, and provided the spawning protocol for future use as an initial larval feed.

21. Production of a procedural guide for the artificial propagation of grouper.

INTRODUCTION

Groupers are commercially important fishes supporting small-scale fisheries in many areas of the world. Capture of groupers is by trawling, hook and line, trap, spear and gill nets. FAO fishery statistics indicate a reported annual worldwide catch in 1988 and 1989 of 85,045 and 80,760 metric tons, respectively. The catch of groupers in Southeast Asian waters alone accounts for approximately 60% of the total catch (Anon., 1989). Although the grouper catch in Southeast Asia by weight is not represented in the top twenty species harvested, it ranks 14th in dollar value (Kohno et al., 1990).

In Palau, commercial export of live groupers from 1984 to 1988 produced an average annual yield ranging between 10 and 15 tons (Kitalong and Oiterong, 1991). Fishing for groupers was largely concentrated in channels and capture was easiest during the large annual spawning aggregations (Johannes, 1981). While seasonal restrictions have been implemented in at least one spawning ground (i.e., since 1980, Ngerumkaol Channel), other areas where groupers aggregate are continuously fished today. Fishermen in Palau are concerned over the dwindling numbers of groupers being caught as exhibited in the catch records of "temekai", over the past five years (Figure 1). The term "temekai" actually is used for a number of groupers (i.e., *Epinephelus fuscoguttatus*, *E. microdon*, *E. maculatus*, *E. tukula*, *E. dyctiphelus*, *E. poecilonotus*, *E. ongus*, *E. merra* and *Anyperdon leucogrammus*) and the catch records do not distinguish between the various species (FitzGerald et al., 1991). This downward trend, however, is particularly noticeable for *E. fuscoguttatus*, even when fishing in the reserve area and during their spawning season. The proposed total fishing ban on grouper will have a serious impact as Palau gains further autonomy and continues to address means of becoming economically independent. In Guam, the grouper populations have been severely over fished for many years and markets import groupers from other areas in the South Pacific (e.g., Chuuk, Palau) to satisfy local demand (FitzGerald, pers. comm.).

The popularity of grouper as a food fish has steadily increased worldwide, resulting in commercial ventures, especially in Southeast Asia, to culture many species. Early culture attempts show that groupers are fast-growing, euryhaline, hardy, disease resistant and therefore profitable. Findings show that grouper culture can be performed in earthen ponds as well as in net cages (Tamaru et al., 1993a). Net cage culture represents an opportunity for expansion of the industry, despite the ever increasing competition for available land space. In Taiwan, groupers fetch a farm gate price ranging from US $15.00 to $20.00/kg (Tamaru et

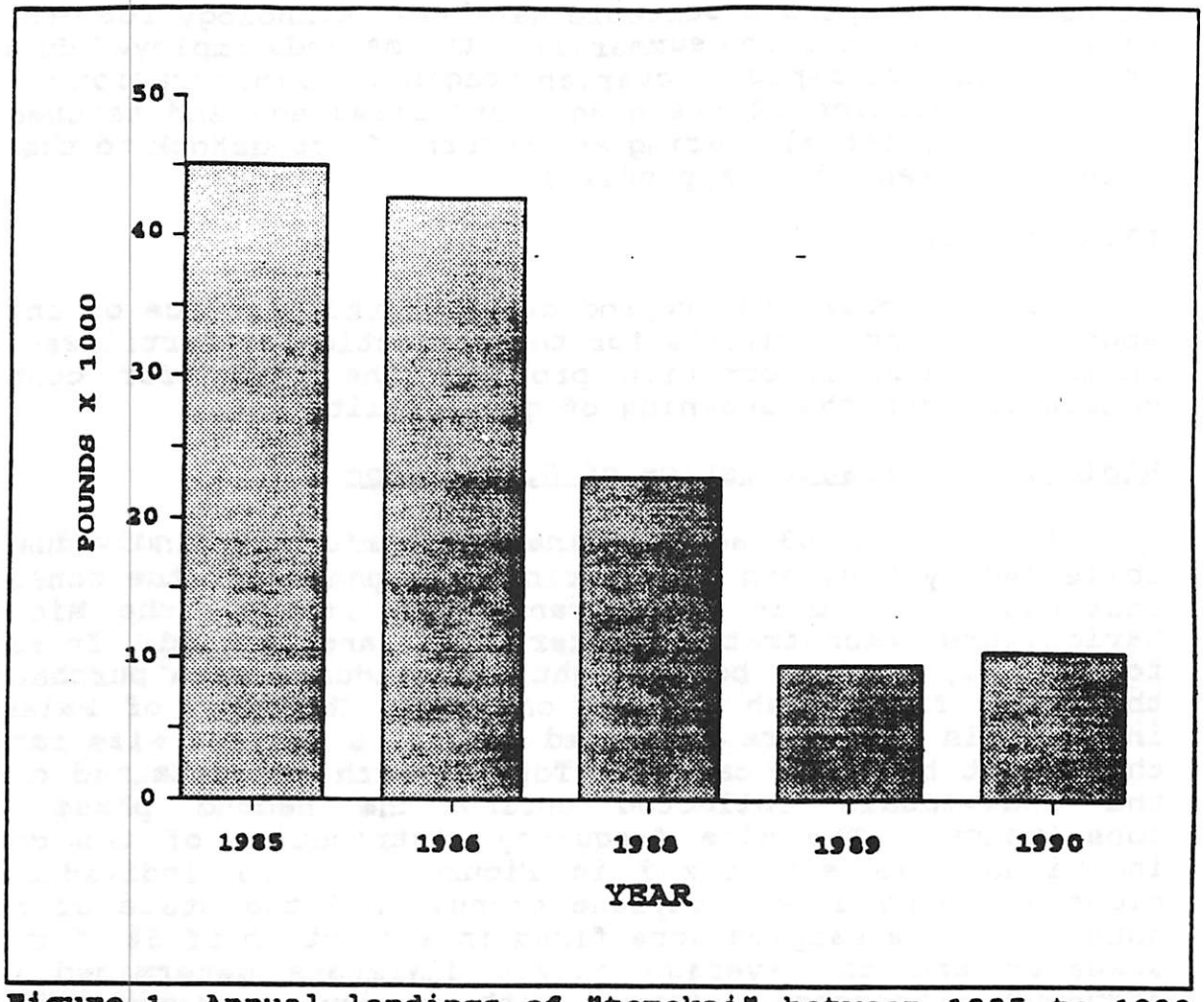


Figure 1. Annual landings of "temekai" between 1985 to 1990 in Palau. Data summarized from Kitalong and Oiterong (1991).

al., 1993a). The grouper culture industry, however, remains dependent on seed from the wild. A single fingerling commands a price of US $2.00 to $3.00 and is currently the highest cost of grouper culture (Tamaru et al., 1993a).

Hatchery technology for the artificial propagation of striped mullet, *Mugil cephalus*, has been in place at GADTC since 1992 as part of the overall objectives to provide seed for the diversification of aquaculture activities in Guam. In keeping with this mission, GADTC has been engaged in adapting the hatchery technology for striped mullet to the production of grouper seed. Seed production of grouper would provide the basis to begin commercial growout and stock enhancement activities. Likewise, the developed technology can be used for the production of grouper species that are conducive to the aquarium trade. The knowledge base and experience of the consultant was used to review, modify, and improve existing hatchery techniques at GADTC with the major

focus on developing a suitable hatchery technology for groupers. A pictorial presentation summarizing the methods employed during this consultancy for capture, ovarian staging, administration of hormone for the induction of spawning, fertilized egg and hatched larvae development, larval rearing and return of broodstock to the capture site is presented in Appendix 1.

REPRODUCTION

Understanding the reproductive characteristics of any target species is a prerequisite for the production of fertilized eggs on demand. Such information provides the basis for controlling maturation and the spawning of good quality eggs.

Biological Characteristics of *E. microdon*

A total of 63 adult *Epinephelus microdon* individuals were collected by hook and line during both phases of the consultancy. Captured individuals were transported live to the Micronesian Mariculture Demonstration Center (MMDC) and weighed. In addition, four (i.e., <0.6 kg body weight) individuals were purchased from the local fresh fish outlets on Koror, Republic of Palau. The individuals that were purchased were of a smaller size range than those that had been caught. Total length was obtained only from the individuals collected during the second phase of the consultancy. The size frequency distribution of the collected individuals is summarized in Figure 2. All individuals were biopsied using a polyethylene cannula and the state of maturity noted. Oocyte samples were fixed in a solution of 5% formalin in seawater and the average oocyte diameters determined using a compound microscope equipped with an ocular micrometer. This method has been validated for use in staging ovarian maturation in female striped mullet (*Mugil cephalus*) and milkfish (*Chanos chanos*) (Shehadeh et al., 1973; Lee et al., 1986). All of the fish caught from the wild were found to be mature, meaning that the females possessed vitellogenic oocytes and from the males, sperm could be extruded by applying pressure to the abdomen or obtained by cannulation. It is obvious from the fish that were examined during July and August that these months mark part of the spawning season for this species. The beginning and ending months of the spawning season remain to be determined.

A length x weight relationship was generated from fish that were caught or purchased during the second phase of the consultancy and is summarized in Figure 3. The following model was found to provide the best fit of the data:

$$BW = 0.067\ (TL) - 1.612$$
$$R^2 = 0.95$$
$$P = <0.001$$

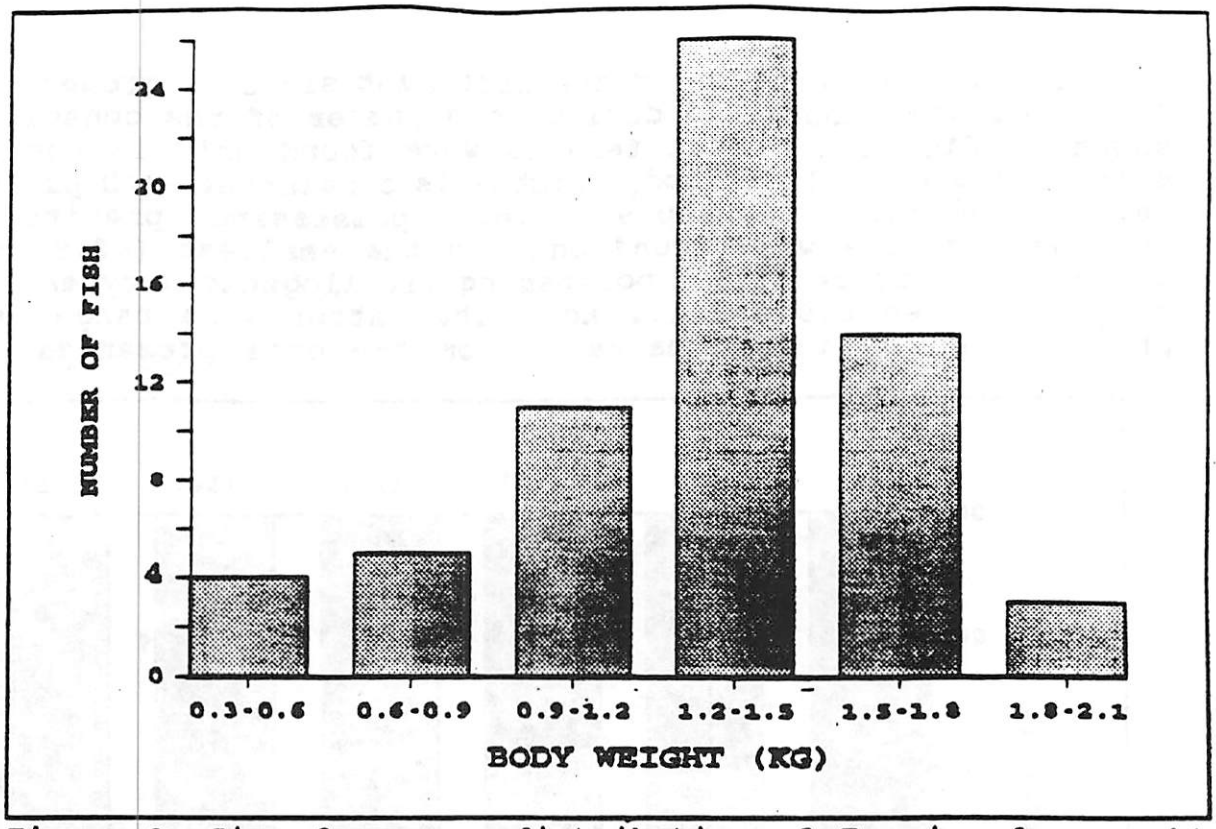


Figure 2. Size frequency distribution of *E. microdon* caught and purchased between July and August 1993.

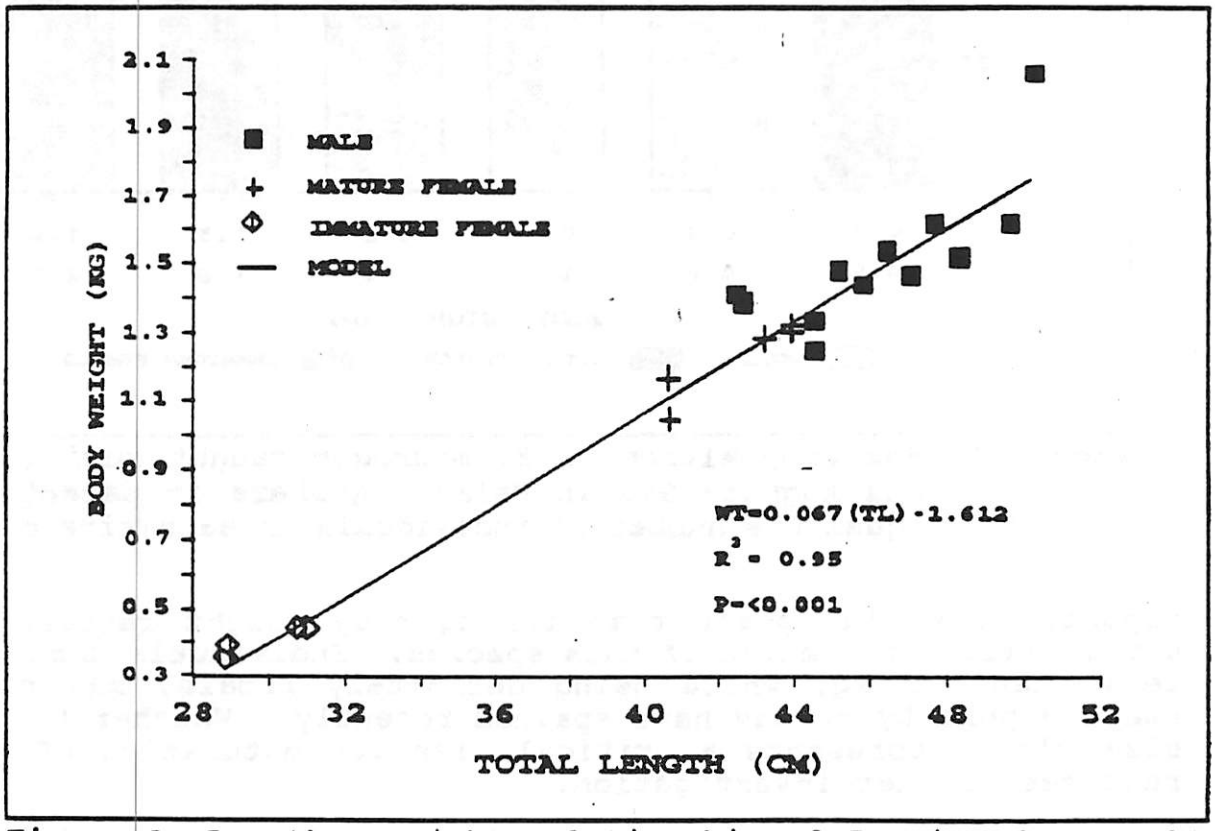


Figure 3. Length x weight relationship of *E. microdon* caught and purchased during the second phase of the consultancy.

The sex composition of the different sizes of groupers (i.e., 0.3 kg classes) obtained during both phases of the consultancy is shown in Figure 4. That females were found only in the smaller size groups (<1.2 kg in body weight) is consistent with protogynous hermaphrodites. Immature (i.e., possessing previtellogenic oocytes) females were found only in the smallest (<0.9 kg) size class while mature (i.e., possessing vitellogenic oocytes) females ranged between 0.9 and 1.2 kg. The latter size range was also predominated by mature males. From the data presented in this

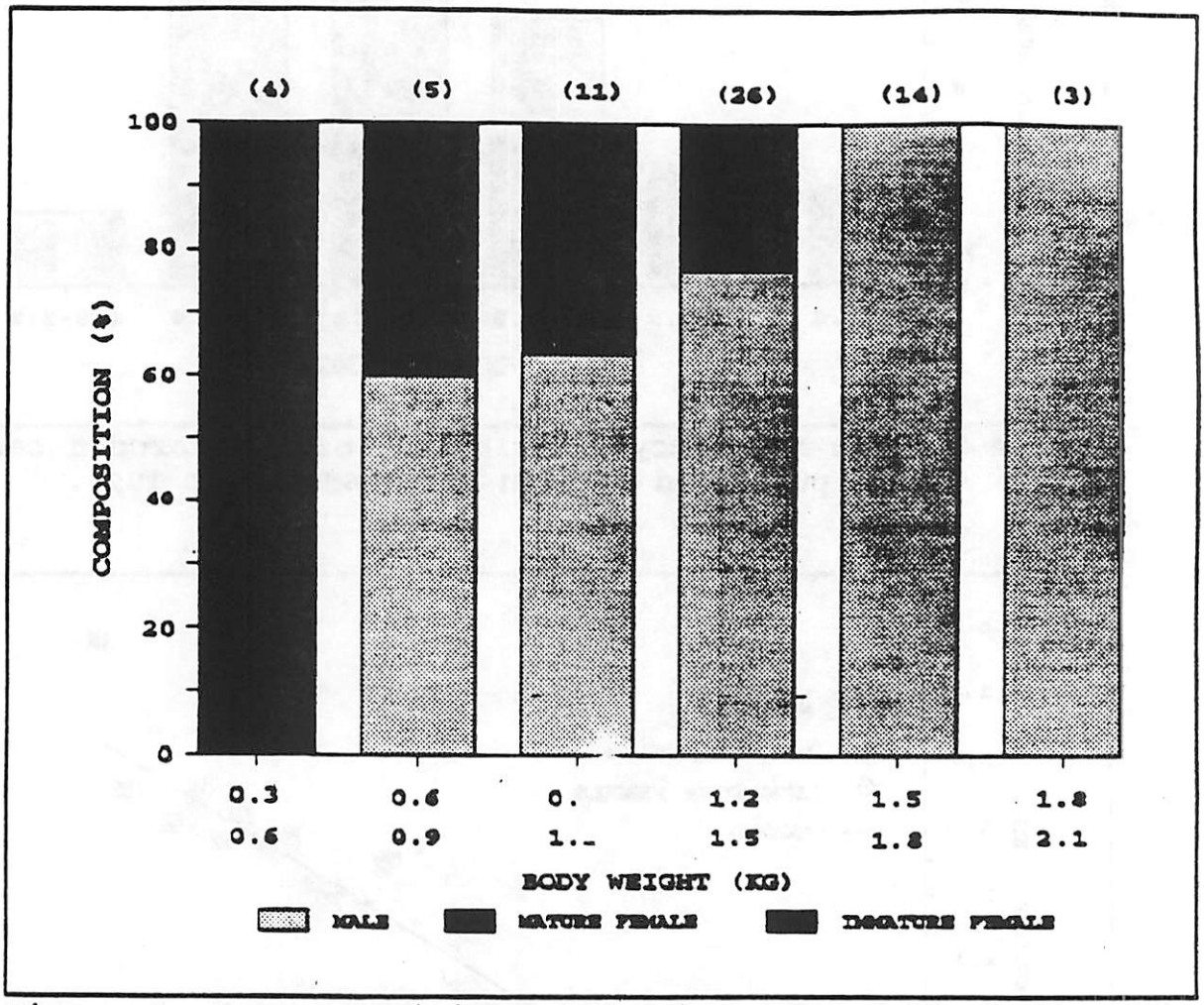


Figure 4. Sex composition of *E. microdon* caught during July and August 1993 in Palau. Numbers in parentheses equal the number of individuals in each size class.

report, it would appear that 1.2 kg body weight represents the upper limit for females of this species. Individuals that weighed less than 0.9 kg, while being definitely female, may not have reached puberty or may have spawned recently. Whether this lower size class represents a critical size for maturation of females requires further investigation.

INDUCTION OF SPAWNING

E. microdon

Females possessing vitellogenic oocytes were injected on July 13, 1993 with a priming injection of 2500 IU HCG/kg body weight between 16:30 and 17:30 hours and placed into a 4000-liter fiberglass tank equipped with running seawater and continuous aeration. Individual females were marked with various cuts to the caudal fin (e.g., TC = top cut, BC = bottom cut, TW = top wedge, and so forth). Five males were chosen at random, administered a single injection of HCG at a dosage of 1000 IU/kg body weight and placed with the females. Ovarian biopsies were repeated on all females 24 hours after the first (priming) injection and oocyte samples preserved. Also at that time, the females were administered a second (resolving) injection of HCG at a dosage of 700 IU/kg and allowed to spawn naturally.

Fertilized eggs from one female were detected to be undergoing first cleavage at 02:30 on July 15, 1993. The water temperature and salinity in the spawning tank were 27°C and 34 ppt, respectively. Over the course of the next two to three hours, the other females were observed spawning. The total number of spawned eggs and the percentage of fertilized eggs were determined from the one spawning tank. The fecundity of individual fish was estimated by dividing the total number of spawned eggs by the number of females (five). The percentage of fertilized eggs was found to be very high (i.e., 99.9%) and the hatch rate from this group spawning was also observed to be very high (i.e., >95%).

Table 1. Summary of spawning trials for *E. microdon* conducted in July 1993 at MMDC.

Fish ID	BW (kg)	Egg Diameter (μm)	First Inject. (IU/kg)	Second Inject. (IU/kg)	# Eggs Spawned	Fert. (%)	Hatch (%)
TCBW	0.79	414	2500	700	$8x10^5$	99.9	>95%
BC	0.87	427	2500	700	$8x10^5$	99.9	>95%
BW	1.03	454	2500	700	$8x10^5$	99.9	>95%
BC	1.11	427	2500	700	$8x10^5$	99.9	>95%
BCBW	1.21	420	2500	700	$8x10^5$	99.9	>95%
TCBC	1.23	415	700	1400	$1.6x10^6$	32.6	0
TC	1.29	369	700	1400	no spawn	-	-

On July 14, 1993, an additional two *E. microdon* females (and marked as TCBC and TC, Table 1) were caught by hook and line and placed with the same group of males that participated in the first

group spawning. Their hormone treatment was initiated on that same day between 16:30 and 17:30 at a dosage of 700 IU/kg followed 24 hours later by a resolving injection of 1400 IU/kg. Spawning was detected for TCBC on July 16, 1993, approximately 12 hours after receiving the second injection. Although TC underwent hydration, she did not spawn.

During the second phase of the consultancy, male and female broodstock were caught by hook and line and treated as described previously. A total of five females were found to possess vitellogenic oocytes and induction of spawning was initiated in three of the females. HCG was administered at 700 IU/kg for the priming injection followed 24 hours later with a resolving injection of 1400 IU/kg. The other two females did not receive any HCG. As with the previous spawning trials, all treated females spawned naturally approximately 12 hours after receiving a second dose of HCG. The two untreated females,"TCBC and BC" although possessing mean oocyte diameters >400 µm, did not spawn (Table 2).

Although all treated females underwent final maturation in response to the hormone treatment as indicated by their abdominal swelling, only one female (i.e., TC, Table 1) did not spawn. It should be noted that she possessed the smallest egg diameters, thus possibly indicating a critical oocyte diameter of >400 µm for hormone induced spawning of this species. To validate this observation, females that possess mean oocyte diameters <400 µm should be obtained and further induced spawning trials attempted. If such individuals continue to complete final maturation but do not spawn naturally, the critical oocyte diameter will have been determined. Future work should also focus on sequentially lower dosages of HCG until the minimum effective dose can be determined.

Table 2. Summary of induction of spawning trials for *E. microdon* in August 1993 at MMDC.

Fish ID	BW (kg)	Oocyte Diameter (µm)	First Inject. (IU/kg)	Second Inject. (IU/kg)	# of Eggs Spawned	Fer (%)	Hatch (%)
TC	1.27	424	700	1400	1.13×10^6	98.5	91.4
TW	1.19	414	700	1400	1.13×10^6	98.3	91.4
BW	1.03	419	700	1400	1.13×10^6	70.1	91.5
TCBC	1.33	438	0	0	no spawn	-	-
BC	1.34	406	0	0	no spawn	-	-

Even though the priming injections used in the second spawning trial were only one third the amount used in the first spawning trial, a similar response was achieved. This would indicate that a lower dosage of HCG can be effective while significantly reducing cost. Until further studies are conducted, it is recommended that

HCG be used exclusively for inducing final maturation and spawning. The advantages of HCG are its standardized potency and ready availability. However, it is higher in cost than alternative hormones (i.e., LHRH-a or pituitary homogenate). Once the lowest dosage of HCG that result in acceptable spawns in both consistency and fertility has been established, alternative hormones can be tested and the most cost-effective hormone treatment formulated. It should be noted that when conducting future work with hormone treatments, all females used should possess mean oocyte diameters of >400 µm so that all females are at a similar state of maturity when treatment is initiated.

An observation consistent during both phases of the consultancy was that each consecutive spawn resulted in a lower percentage of fertilized eggs (30.6% and 70.1%, Tables 1 and 2, respectively). This was particularly evident during the second phase when the spawning of individual females was directly observed, indicating a depletion of sperm in males taking part in successive spawns. It is therefore recommended that to maximize the percentage of fertilized eggs from each spawn, the composition of broodstock be at a 2:1 male to female ratio. The males would participate in only one spawning event.

Table 3. Summary of hormone treatment for maturation of *E.microdon* males.

Fish ID	BW (kg)	Initial State of Maturity	Dosage of HCG (IU/kg)	State of Maturation
Trial 1				
Male 1	n.a.	cann. milt	1000	milting
Male 2	n.a.	cann. milt	1000	milting
Male 3	n.a.	cann. milt	1000	milting
Male 4	n.a.	cann. milt	1000	milting
Male 5	n.a.	cann. milt	1000	milting
Trial 2				
Male 1	2.05	cann. milt	500	milting
Male 2	1.46	cann. milt	500	milting
Male 3	1.45	cann. milt	500	milting
Male 4	1.24	cann. milt	500	milting
Male 5	1.51	cann. milt	500	milting

n.a. = data not available
cann. milt. = milt obtained with a cannula

All males examined 24 hours after receiving HCG were found to be in a running ripe condition, i.e., milt could be easily extruded by applying slight pressure to their abdomen (Table 3). It is concluded that the hormone treatment was effective in inducing spermiation at all dosages tested. Modifications in the dosage of

HCG used in induction of spawning require further investigation to determine the minimum dosage effective for natural spawning. Future work should also examine the effectiveness of 17 α-methyltestosterone (17-MT) to increase the production of sperm. Chronic 17-MT treatment at a dosage of 5 µg/g body weight has been demonstrated to be effective in increasing the frequency of spawns in which striped mullet males could participate without a decrease in the percentage of fertilized eggs (Lee et al., 1992a).

Upon completion of the spawning trials, some of the captured individuals were kept at MMDC and placed in an exhibit for public display. The remaining individuals were returned to Ngerumkaol Channel.

E. fuscoguttatus

A total of nine female and male *E. fuscoguttatus* were caught and examined on July 13, 1993, and treated as described for *E. microdon* with the exception of the dosage of HCG administered. An ovarian biopsy performed on female "BC" at approximately 04:30 on

Table 4. Summary of spawning trial for *E. fuscoguttatus*.

Fish ID	BW (kg)	Oocyte Diameter (µm)	First Inject. (IU/kg)	Second Inject. (IU/kg)	# Eggs Spawned	Fert. (%)	Hatch (%)
TC	3.21	432	700	1400	ovulated	-	-
BC	3.35	435	700	1400	1.5×10^6	81.0	52.8
TW	4.52	411	700	1400	ovulated	-	-
BW	4.35	400	250	500	ovulated	-	-
TCBC	3.83	407	250	500	ovulated	-	-
NC	2.69	417	0	0	-	-	-
NC	4.35	464	0	0	-	-	-
		Maturity of Testes			Maturity of Testes		
MALE NC MALE DC MALE/MMDC	6.15 6.15 10.30	cann.milt cann.milt negative	700 700 none		milting milting		

cann. milt = cannulated milt, not running ripe
negative = no maturation

July 15, 1993, found her to have ovulated completely. She was then strip spawned and the eggs were manually fertilized at 05:05 using the dry method (Appendix 2, Steps 1-6). The resulting

fecundity, fertilization, and percent hatch of the spawned eggs are presented in Table 4. Females identified as TC, TW, BW and TCBC were allowed to complete final maturation in the same tank. However, they did not spawn and the ovulated eggs were manually stripped on the afternoon of July 16, 1993, as the ovulated oocytes by this time period were overripe and nonviable. From past experience with striped mullet females, if the overripe oocytes are not removed from the females they will ultimately die. The two untreated females, "NC", possessed vitellogenic oocytes (i.e., did not ovulate) by the end of the trial.

Males of this species were difficult to obtain from the wild and only two individuals were caught during the entire consultancy. Both of these were administered HCG as described for *E. microdon* and were found to respond in similar fashion. In addition, a single male *E. fuscoguttatus* held captive at MMDC for public exhibit was examined for milt. The gonadal biopsy, however, was negative.

Although, all treated females responded by undergoing final maturation, natural spawning was not achieved in this species. The strip spawn of the one female resulted in fertilized eggs that developed and hatched normally indicating that the proper state of maturity had been achieved. It is recommended that future natural spawning trials for *E. fuscoguttatus* be conducted in a larger and deeper holding facility. The same recommendations for future work with *E. microdon* regarding dosage and use of alternative hormones apply to *E. fuscoguttatus*.

SPAWNING BEHAVIOR

Hormonal induction of final maturation in *E.microdon* resulted in the spontaneous release of eggs from the females and immediate fertilization by the males. The changes in behavior during the course of the hormone treatment, including the spawning event, were photographed (Appendix 3, Plate 1-3). Initially, (i.e., after the priming injection) both males and females were relatively inactive and observed resting on the bottom or in the corners of the spawning tank. By the second injection, however, the abdomens of the treated females were noticeably distended with the distention becoming more apparent as spawning approached. With the increase in abdominal swelling the cloaca began to protrude. Approximately five to eight hours after the second injection, the males were noticeably closer to the treated females and were observed engaging in physical contact in the form of nudging. As each female approached spawning, she began to swim short distances around the tank. During these brief periods of swimming activity, the males would collectively rush and surround her. This activity immediately preceded spawning. The spawning event concluded with males (2-3 individuals) and the female often times breaking the surface of the water. The eggs were usually released in two or three abrupt bursts and were collected in an egg collector coupled to the drain of the spawning tank (Appendix 3, Plate 4).

TEMPORAL CHANGES OF GROUPER OOCYTES DURING FINAL MATURATION

Ovarian biopsies performed during the induction of final maturation and spawning for both species of grouper were examined in order to: 1) evaluate whether the state of maturity at which hormone treatment was induced was appropriate, and 2) identify the stage at which the strip spawn method could be employed. The temporal changes, *in vivo*, in the size frequency distribution of oocytes obtained from *E. fuscoguttatus* (marked "BC") as she underwent hormonal induction of final maturation are presented in Figure 5. These changes were observed to be the same for *E. microdon*.

Morphological changes, *in vivo*, in oocytes were also observed for both species and photographed through a compound microscope (Appendix 4, Plates A-D). In summary, the changes observed in these groupers were found to be characteristic of those observed for other fish species, e.g., striped mullet (Tamaru et al., 1991b). From these observations and the high fertilization and hatching of eggs obtained during this consultancy, it can be safely assumed that oocytes >400 µm in size are receptive to hormonal induction of spawning.

When utilizing the strip-spawn method, a common question is when is the fish ready to be stripped of eggs? A prerequisite to stripping is that the female complete ovulation. She can be staged by conducting an ovarian biopsy and examination of the oocytes. A photograph of an ovulated oocyte is presented in Appendix 4, Plate C. Note the similarity between this oocyte and that of the spawned egg (Appendix 4, Plate D). When the majority (>95%) of retrieved oocytes have ovulated, the female can be stripped and the eggs fertilized. The percentage of oocytes that have ovulated can be easily determined by holding the polyethylene cannula up to a light source. The ovulated oocytes will resemble a string of small glass beads and can easily be distinguished from vitellogenic oocytes (Appendix 4, Plates A and B) by their transparent appearance.

Future work should continue to focus on achieving natural spawns from *E. fuscoguttatus*.

FATTY ACID PROFILES OF SPAWNED EGGS

The fatty acid profiles of spawned eggs from the first spawning trial of five *E. microdon* females and from the single *E. fuscoguttatus* spawn are summarized in Table 5. The analyses were conducted by Dr. Harry Ako, Department of Environmental Biochemistry, University of Hawaii at Manoa.

For comparison, the fatty acid profiles of spawned eggs from other marine teleosts are presented in Table 6. The total fatty

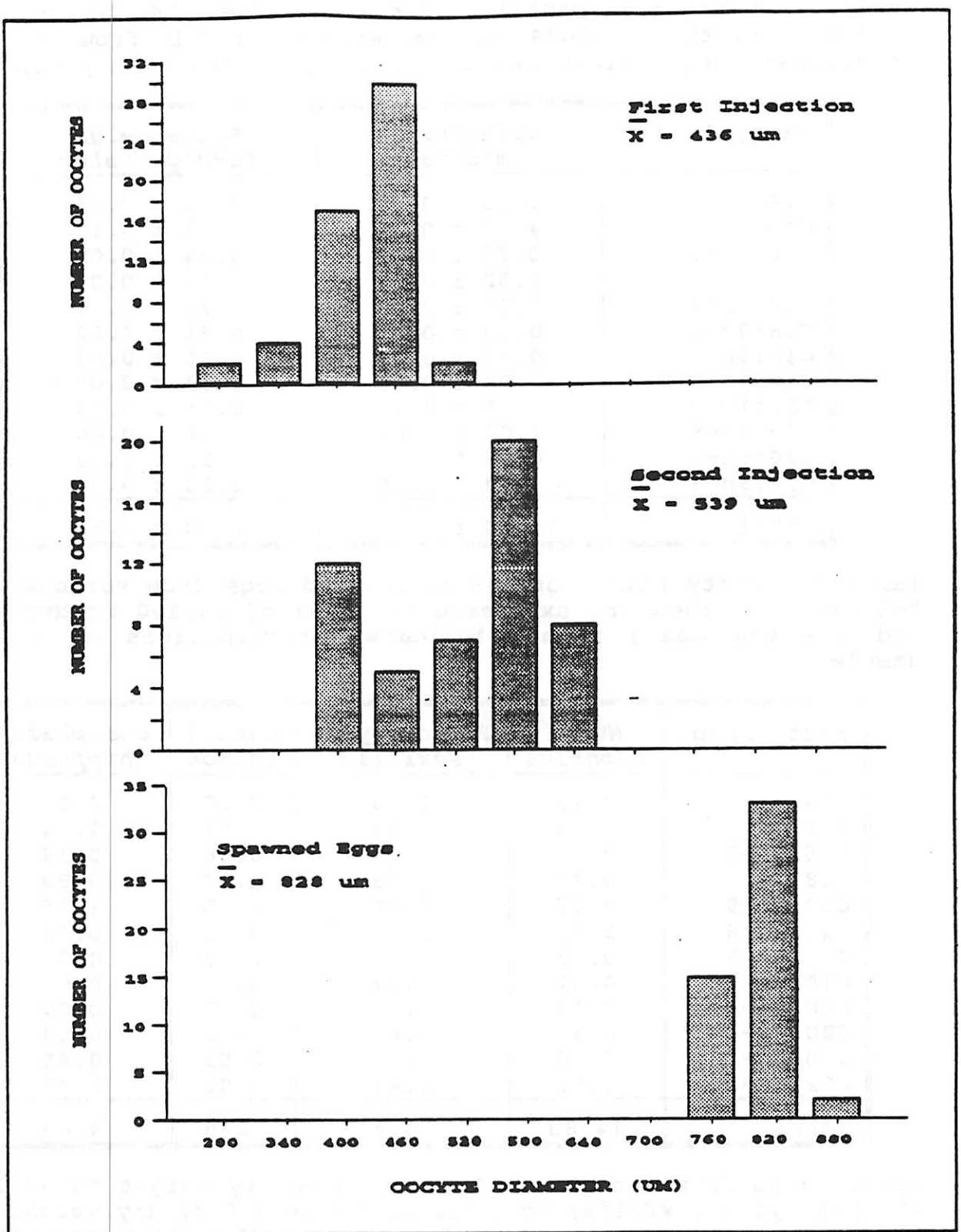


Figure 5. Changes in the oocyte diameters of "BC" female *E. fuscoguttatus* undergoing induction of spawning.

Table 5. Fatty acid profiles of spawned eggs from *E.microdon* and *E. fuscoguttatus*. Values are the means ± 95% C.I. from triplicate determinations. Values are presented in mg/100 mg dry weight.

Fatty Acid	*Epinephelus microdon*	*Epinephelus fuscoguttatus*
C14	0.45 ± 0.03	0.32 ± 0.05
C16	4.67 ± 0.19	3.31 ± 0.12
C16:1n-7	0.75 ± 0.02	0.64 ± 0.05
C18	1.52 ± 0.09	1.14 ± 0.01
C18:1n-9	2.77 ± 0.08	1.73 ± 0.10
C18:2n-6	0.25 ± 0.02	0.24 ± 0.02
C18:3n-3	0.12 ± 0.03	0.15 ± 0.01
C18:4n-3	0.02 ± 0.00	0.03 ± 0.00
C20:1n-9	0.05 ± 0.01	0.06 ± 0.00
C20:4n-6	1.00 ± 0.04	0.74 ± 0.00
C20:5n-3	0.29 ± 0.01	0.32 ± 0.06
C22:6n-3	2.18 ± 0.05	2.10 ± 0.03
Total	14.10 ± 0.23	11.10 ± 0.78

Table 6. Fatty acid profiles of spawned eggs from various marine teleosts. Values are expressed in terms of mg/100 mg dry weight and are the means from triplicate determinations of a single sample.

Fatty Acid	*Mugil cephalus*	*Polydactylus sexfilis*	*Chanos chanos*	*Coryphaena hippurus*
C14	0.21	1.06	0.10	0.14
C16	1.64	4.63	3.57	1.74
C16:1n-7	3.21	3.00	0.36	0.18
C18	0.42	1.06	1.13	0.99
C18:1n-9	4.27	5.28	2.59	1.35
C18:2n-6	2.15	1.19	0.36	0.78
C18:3n-3	0.15	0.15	0.08	0.06
C18:4n-3	0.07	0.32	0.02	0.02
C20:1n-9	0.13	0.27	0.17	0.09
C20:4n-6	0.40	0.60	0.63	0.59
C20:5n-3	0.58	1.41	0.53	0.65
C22:6n-3	1.51	4.47	3.02	1.77
Total	14.80	23.4	12.6	8.40

acids range from a high of 23.4 mg/100 mg dry weight found in moi (*Polydactylus sexfilis*) to a low of 8.4 mg/100 mg dry weight found in mahi mahi (*Coryphaena hippurus*). When the nutritional requirements of a target species are unknown, one strategy to determine them is to attempt to mimic the fatty acid profile of the

spawned eggs. It should be noted, however, that the spawned eggs from fish in captivity can be profoundly influenced by their respective diets. Values obtained from the grouper eggs in the current study are from wild caught individuals and thus can be used as a first approximation. In addition, the values obtained for groupers in the current study can serve as a standard for comparison against spawned eggs obtained from captive broodstock in the event that egg quality ever becomes an issue.

ACTIVATION OF GROUPER SPERM AT VARIOUS SALINITIES

Hormone treated *E. microdon* and *E. fuscoguttatus* males were examined on July 16, 1993, for possession of milt. A sample of sperm was collected from a single male of each species with a 5 cc syringe and capped with a hypodermic needle. The sperm was then activated at several different salinities to determine the salinity and respective osmotic pressure at which sperm activate. Solutions with salinities higher than 35 ppt were prepared by mixing seawater with Instant Ocean Sea Salt. Salinities below 35 ppt were prepared by serial dilutions of seawater with fresh water. Osmotic pressure at the respective salinities were calculated using the formula described by Lee et al., (1992b):.

$$mOsm/kg = (29.572 * \text{salinity ppt}) - 8.859$$

The results of the experiment (Table 7) indicate that salinities below 27 ppt are not conducive for activation of sperm from these species of groupers.

Table 7. Activation of *E. microdon* and *E. fuscoguttatus* sperm at various salinities.

Salinity (ppt)	Osmotic Pressure mOsm/kg	*E. fuscoguttatus*	*E. microdon*
5	139.0	-	-
7	198.1	-	-
9	257.3	-	-
12	346.0	-	-
15	434.7	-	-
20	582.6	- (weak)	- (weak)
27	789.6	+	+
35	1026.2	+	+
47	1381.0	+	+
62	1824.6	+	+
72	2120.3	+	+
95	2800.5	+	+

The data define the critical ranges in salinities in which fertilization can take place. The results of the experiment also

provide basic information regarding the osmolarity at which sperm from both species of grouper are activated. It has been demonstrated that sperm from marine fishes are activated in response to osmotic pressure rather than the presence of specific ions (Morisawa, 1985; Lee et al., 1992b). This information was used to test various solutions as sperm extenders for use in future work involving strip spawning.

SPERM EXTENDERS

It is hypothesized that sperm extenders at low (i.e., 5°C) temperatures work either by suspending the activity of sperm in a physiologically suitable (i.e., osmotically) medium or by providing an adequate supply of energy to keep the sperm active for a prolonged period of time. The energy source is usually in the form of sugars. The mechanism of action varies with the species but can be initially tested by mixing raw milt with various solutions containing sugar at varying concentrations.

On August 14, 1993, milt from an *E.microdon* male that had been injected with HCG was obtained by gentle stripping and collection with a 12 cc syringe. The milt was then immediately capped with a hypodermic needle. Four solutions (i.e., fish ringers, 1% glucose, 5% glucose, and 10% glucose dissolved in fish ringers) were tested for their effectiveness as sperm extenders. The fish ringer consisted of 1.35 g NaCl, 0.06 g KCl, 0.02 g $NaHCO_3$, 0.025 g $CaCl_2$, 0.035 g $MgCl_2$ in 100 ml distilled water and 50 IU/ml penicillin, as described by Chao et al., (1975). The glucose solutions were prepared using α-D-glucose dissolved in fish ringers in a weight/volume ratio. The osmotic pressure (mOsm/kg) of the extenders was measured using a Wescor 5100 C Vapor Pressure osmometer. Equal volumes of raw milt and the respective solutions were mixed in separate 12 cc hypodermic syringes and capped with hypodermic needles. All samples were stored at 5°C. Sperm activation was initiated by adding a drop of seawater to a drop of milt or milt + extender placed on a microscope slide. A coverslip was then used to combine the two drops. Activity was qualitatively scored (ranging from +++ = active to - = no activity, Table 8) at various time intervals as observed under a compound microscope.

Sperm could be activated in all solutions five days after the initiation of the experiment. By Day 9 of the experiment only sperm obtained from the raw milt exhibited signs of activity. The solutions containing glucose have been reported to extend the life of sperm from striped mullet up to 21 days when stored at 5°C. However, the fact that sperm from grouper remained viable for at least 5 days when kept at 5°C is valuable information for future spawning trials. The fertility of the extended sperm still requires further investigation. Future work should focus on the use of other reagents (glycerin, DMSO) reportedly effective in extending the life of sperm in other species.

Table 8. Summary of various sperm extenders on maintaining sperm of *E. microdon* at 5°C.

Treatment	Osmotic Pressure mOsm/kg	8/14	8/15	8/16	8/19	8/23
Raw Milt	n.a.	+++	+++	+++	+++	+
Fish Ringer	420	-	-	++	++	-
1% Glucose	462	+	+++	+++	++	-
5% Glucose	700	+++	+++	+++	++	-
10% Glucose	986	+++	+++	+++	+	-

n.a. = not available.

EMBRYONIC DEVELOPMENT OF GROUPER LARVAE

The temporal changes in embryonic development for both species of groupers were obtained by photographing various stages of development at approximately one hour intervals through a compound microscope. The stages of development and time intervals for *E. fuscoguttatus* are summarized in Table 9. Photomicrographs for each stage of development are presented in Appendix 4, Plate A-P.

Table 9. Temporal changes in oocytes undergoing final maturation, and developmental series of eggs from the strip spawn of "BC" *E. fuscoguttatus* at 27°C and 34 ppt.

Plate Number	Date	Time	Stage of Development
A	7/14	17:04	Vitellogenic Oocyte, First Injection
B	7/15	17:41	Vitellogenic Oocyte, Second Injection
C	7/16	05:30	Ovulated Oocyte
D	7/16	06:00	Spawned Egg
E	7/16	06:30	First Cleavage, Two-cell Stage
F	7/16	06:45	Second Cleavage, Four-cell Stage
G	7/16	07:00	Third Cleavage, Eight-cell Stage
H	7/16	09:00	Multicell Stage
I	7/16	10:00	Blastula, Top view
J	7/16	10:00	Blastula, Side view
K	7/16	10:30	Early Gastrula
L	7/16	11:15	Gastrulation
M	7/16	12:15	Embryo Formation
N	7/16	16:15	Somite Stage
O	7/16	22:00	Late Embryo, Muscle Twitching
P	7/17	24:00	Newly Hatched Embryo

To determine the quality of a spawn, the investigator must be able to distinguish between "good" and "bad" eggs. Cleavage greatly simplifies the identification of "good" eggs. "Bad" or unfertilized eggs, however, can be more difficult to detect especially when poor fertilization is a result of the male. Two examples of "bad" eggs are presented in Appendix 4, Plates Q and R.

Development was found to be similar for both species in regard to egg size and rate of development. Hatching took place 18.5 hours after fertilization of the eggs which is consistent with that reported for this species (Chao et al., 1991). The rate of development and time of hatching after fertilization for *E. microdon* was found to be comparable.

Future studies should focus on the effects of salinity and temperature on the rate of embryonic development to define the optimal conditions for hatching.

EGG TRANSPORT

Fertilized *E. microdon* and *E. fuscoguttatus* eggs were packed at a density of 5000 eggs/l and transported via airline to GADTC to conduct larval rearing trials. This density has been successfully used to transport striped mullet and milkfish eggs from Hawaii to Guam. Because the distance between Palau and Guam is much shorter, an experiment simulating transport conditions (Figure 6) was conducted using *E. microdon* eggs to determine if spawned eggs could be transported at a higher density without loss of viability. Fertilized eggs at various densities were stocked into plastic bags containing one liter of seawater at a salinity elevated to 40 ppt (by adding Instant Ocean Sea Salt). The bags were filled with oxygen, sealed with rubber bands and placed into a styrofoam cooler for eight hours. All treatments were carried out in triplicate. After the elapsed time, 100 eggs from each bag were placed into 1-liter beakers filled with seawater at 34 ppt and allowed to hatch. The number of hatched larvae was then determined and the data summarized. The results of the experiment indicate that grouper eggs can be transported from Palau to Guam at a density as high as 20,000 eggs/l. Eggs transported at this density would obviously result in a significant savings in cost. Future work should include studies to determine if fertilized grouper eggs can be shipped from Palau to Guam at even higher (>20,000 eggs/l) densities and retain their viability.

LARVAL REARING

Larval rearing includes the production of live feeds at sufficient quantity and quality for larval survival and growth. The production of live feeds includes phytoplankton and rotifer culture, copepod production, and the production and enrichment of *Artemia* nauplii. Once live feeds can be produced on demand, the optimum densities of feed and larvae and the temporal changes in

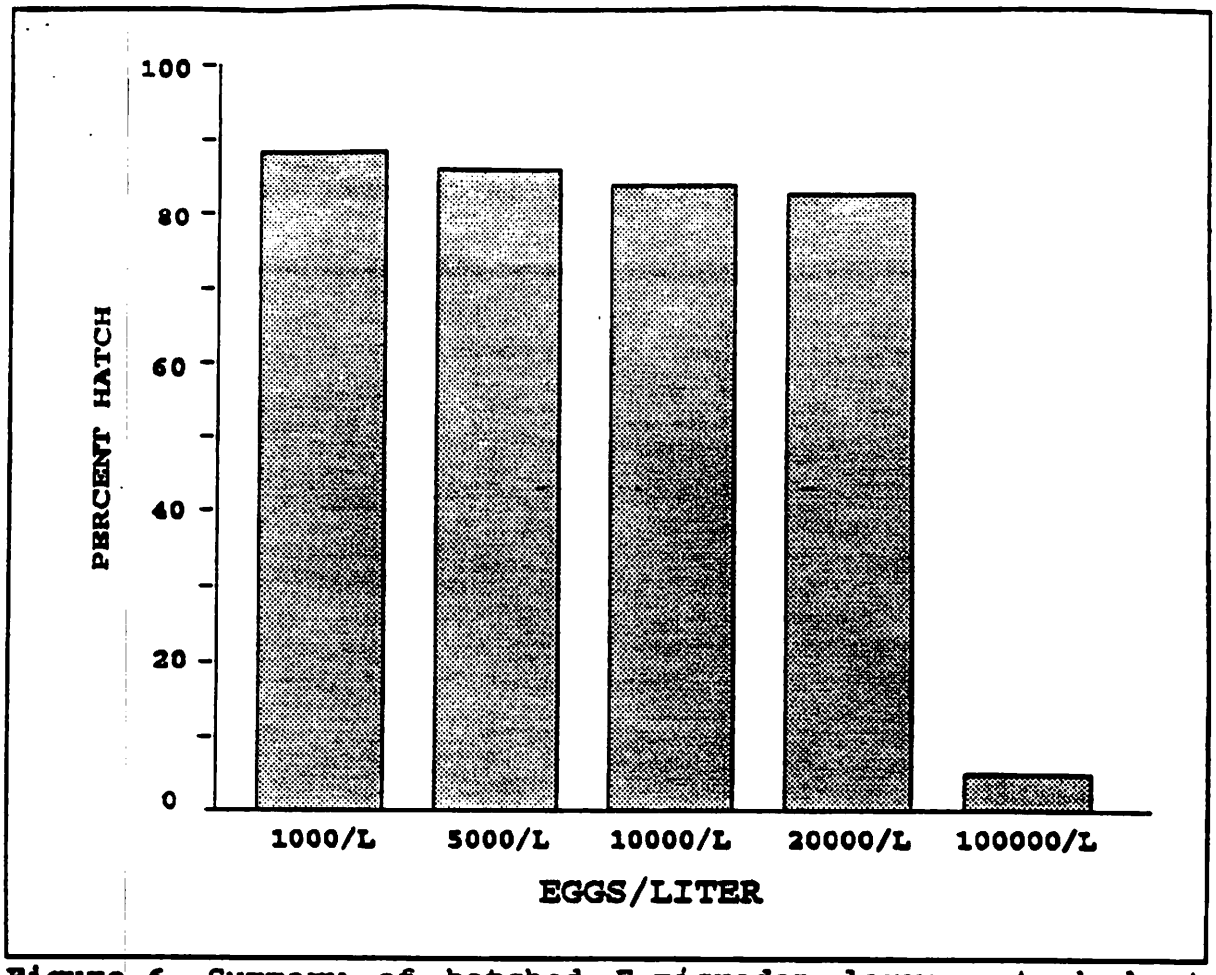


Figure 6. Summary of hatched *E.microdon* larvae stocked at various egg densities under simulated transport conditions.

preference for a particular feed that occur as the larvae grow need to be established.

One of the reasons that groupers have not enjoyed the success of other marine species under culture is that grouper larvae are one of the smallest encountered in mariculture and thus require a small (70-100 μm) initial feed. The relationship between spawned egg diameters and total length of larvae at hatching of several other fish species under culture is presented in Figure 7. To further illustrate this point, the morphological changes that occur during the first four days posthatching of *E. fuscoguttatus* are pictured in Appendix 5, Plate 1-6. The mouth of the grouper larvae is not fully developed until Day 2 or Day 3 posthatching. Measurements from four-day-old larvae indicate a mouth size of 150 μm when fully extended (Appendix 5, Plate 6). This is smaller than the average size of the S-type rotifer (170 μm, lorica length) currently cultured at GADTC. Finding a suitable initial feed is a major hurdle to be overcome in the development of a protocol for the larval rearing of grouper.

LIVE FEEDS PRODUCTION AT GADTC

Each live feed cultured at GADTC and their methods of culture were reviewed and modified as necessary to meet the requirements anticipated for the hatchery production of grouper. In addition, oyster trochophores were tested as an initial feed for grouper larvae.

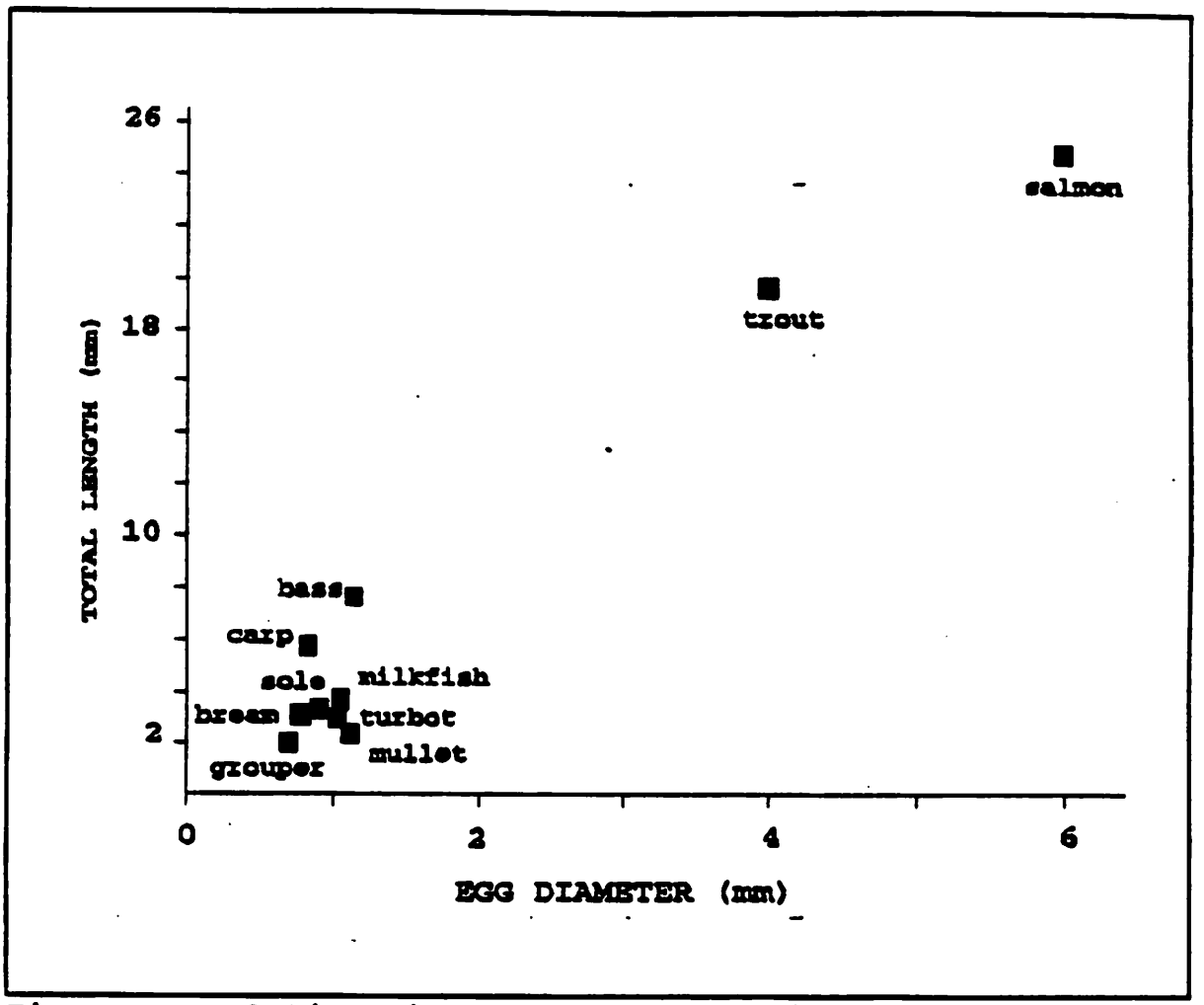


Figure 7. Relationship of spawned egg diameter versus total length of hatched larvae from various species under culture.

Oyster Trochophores

One objective of this consultant was to determine the feasibility of oyster trochophores as an initial food for grouper larvae at GADTC. Cryopreserved oyster trochophores were purchased from MTL Biotech, Ltd., Victoria, B.C., Canada, for this purpose (see Appendix 6 for more information regarding the preparation and handling of this product). In areas such as Guam where oysters are difficult to obtain and spawn, this product allows for direct use

of this live feed with minimum preparation. A sample has been submitted for fatty acid analysis. The results of the larval-rearing trials using oyster trochophores are presented in a later section. A photomicrograph of a rotifer and oyster trochophores is presented in Appendix 6, Plate 1, providing a size comparison. It is apparent that the size of the oyster trochophore is suitable for first-feeding grouper larvae. The densities necessary for adequate larval survival have yet to be determined. Once the optimal densities have been determined, the results should be evaluated at commercial scale so that the economic feasibility of using oyster trochophores as a feed can be established.

Phytoplankton Production

Production of the phytoplankton, *Nannochloropsis oculata*, has been ongoing at GADTC since 1991. This particular species of phytoplankton is vital to the development a hatchery technology for grouper because of its high fat content, in particular, the long chain polyunsaturated fatty acids C20:5n-3 and C22:6n-3. At present, the facility can produce 4000 liters/day at a density >10 x 10^6 cells/ml, or enough to supply their current needs. However, expansion to larger larval rearing systems will require an increase in volume of production and additional staff.

N. oculata is currently cultured at 28 ppt by the batch culture system utilizing a commercial preparation of nutrients (i.e., Mass Pack, Florida Aqua Farms, Inc.). No improvements are currently suggested for the production of this species at GADTC. The only recommendation is that an increase in volume of production be considered to meet the projected expansion of larval marine fish culture. This would require additional tank space and aeration capacity. GADTC has purchased materials for the construction of 10 additional 20,000-liter round fiberglass tanks that are scheduled to be installed in 1994.

Rotifer Production

Currently, the S-type rotifer (*Brachionus plicatilis*) is cultured at GADTC by the batch culture system described by Lubzens (1987) and referred to here as the "Old Method." The phytoplankton, *N. oculata* is used exclusively as a feed source. Daily production ranges from 100 to 200 x 10^6 rotifers/day. A modified version of batch culture system called the "New Method" was introduced by the consultant to improve daily rotifer production as well as the nutritional profile of the rotifers. A comparison of both culture systems is schematically presented in Figure 8. Production results from both systems are summarized in Table 10. The "New Method" produces a significantly higher number of rotifers per day. This is accomplished by increasing the amount of food available to the rotifers resulting in an increase in the rate of reproduction as depicted in the increased egg ratio values.

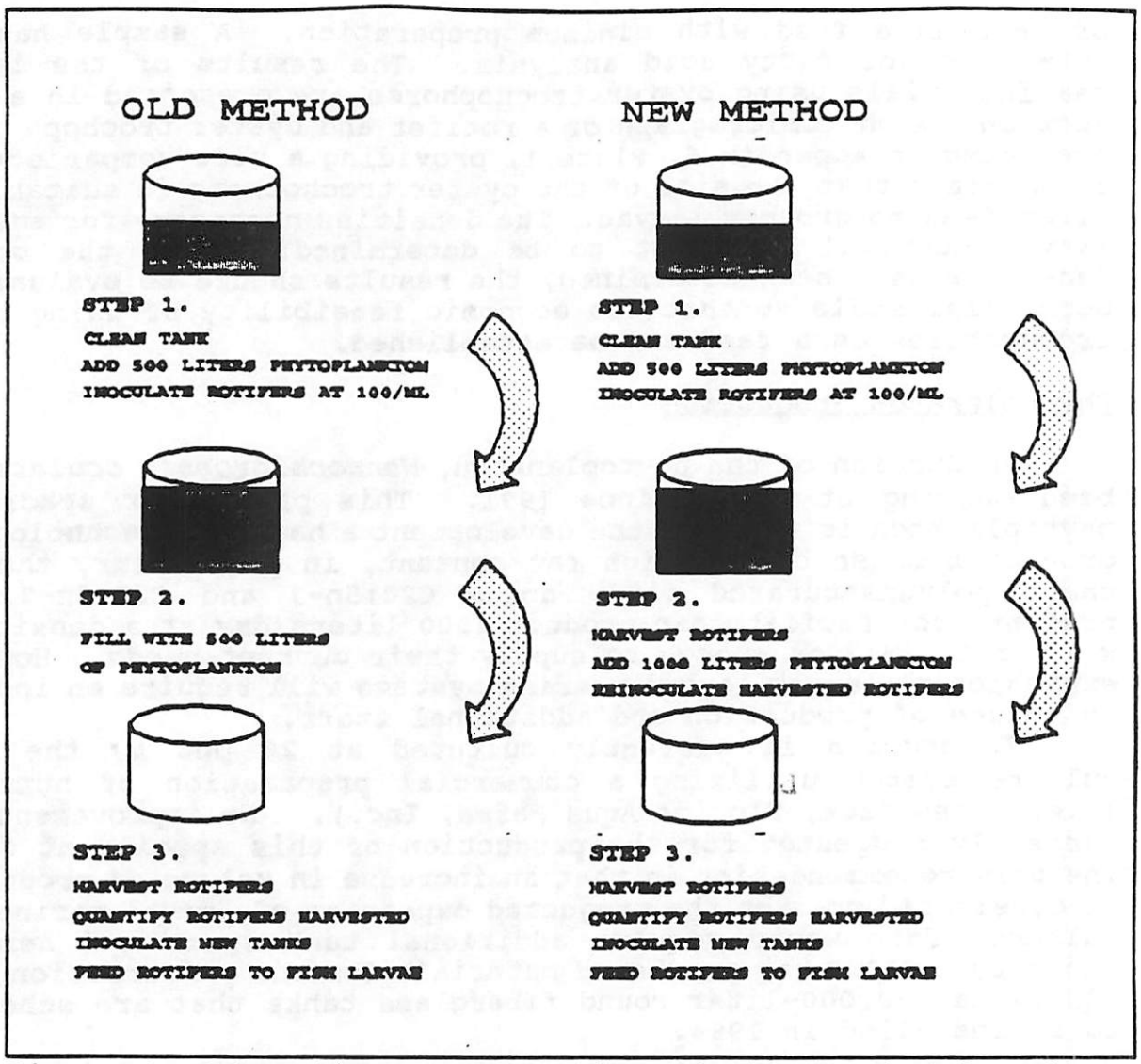


Figure 8. Schematic representation of the two batch culture methods for culturing rotifers as GADTC.

Table 10. Summary of rotifer production at GADTC using different batch culture methods.

Old Method		New Method	
Trial 1 Daily Production	160×10^6	Trial 1 Daily Production	220×10^6
Trial 2 Daily Production Egg Ratio	143×10^6 1.0 %	Trial 2 Daily Production Egg Ratio	183×10^6 17.6%

Because of the increase in the phytoplankton consumed by the rotifers, the amount of fat present in the rotifers also increases

(Table 11). The fatty acid profiles of rotifers produced by the respective methods were analyzed by Dr. Harry Ako of the Department of Environmental Biochemistry at the University of Hawaii at Manoa. Rotifers cultured by the "Old Method" show fatty acid values consistent with previous reports (Tamaru et al., 1991). With the exception of four fatty acids, all others significantly ($P<0.05$, t-test) increased when cultured by the "New Method." Among the fatty acids that exhibited an increase are the long chain fatty acids C20:5n-3 and C22:6n-3 or EPA and DHA, respectively. Both have been found to be essential for survival, growth and stress resistance in larval marine fish (Sorgeloos et al., 1991). The "New Method" thus not only increases daily rotifer production, but results in a rotifer with a higher nutritional value as well.

A newly developed product from Provesta Corporation, Oklahoma, (i.e., Microfeast 100) tested to boost rotifer fatty acid profiles reportedly results in the highest EPA and DHA values. The values are presented in Table 11 for comparison. A modification of the "Old Method" by the addition of this product was tested at GADTC in 20-liter buckets. The combination differs from the "Old Method" by the addition of 0.6 g/1 x 10^6 rotifers which were given in two separate feedings on the second day of the culture process. A side-by-side comparison of all three methods is presented in Table 12.

Table 11. Comparison of fatty acid profiles from rotifers produced at GADTC by different batch culture methods. Values are the mean ± 95% CI from three separate determinations and are expressed in terms of mg/100 mg dry weight.

Fatty Acids	Old Method	New Method	Microfeast 100
C14	0.15 ± 0.14	0.31 ± 0.16	0.17
C16	1.64 ± 0.18	2.49 ± 0.22	0.16
C16:1n-7	0.48 ± 0.05	0.79 ± 0.16	0.20
C18	0.36 ± 0.10	0.57 ± 0.44	0.44
C18:1n-9	0.48 ± 0.09	0.83 ± 0.46	1.09
C18:2n-6	0.33 ± 0.04	0.51 ± 0.25	2.11
C18:3n-3	0.02 ± 0.02	0.04 ± 0.02	0.44
C18:4n-3	0.02 ± 0.02	0.01 ± 0.00	0.16
C20:1n-9	0.10 ± 0.01	0.14 ± 0.07	0.29
C20:4n-6	0.36 ± 0.02	0.63 ± 0.22	0.14
C20:5n-3	0.76 ± 0.05	1.11 ± 0.23	1.82
C22:1n-11	0.03 ± 0.01	0.04 ± 0.03	0.14
C22:6n-3	0.48 ± 0.04	0.77 ± 0.28	1.57
Total Fatty Acids	5.22 ± 0.57	8.09 ± 0.74	9.82

Table 12. Comparison of production and fertility of rotifers cultured by three different methods.

	Old Method	New Method	Combination
Trial 1			
Daily Production	3.2×10^6	3.5×10^6	4.3×10^6
Egg Ratio	7.6%	25.3%	16.8%
Trial 2			
Daily Production	2.2×10^6	2.3×10^6	2.1×10^6
Egg Ratio	23.0%	38.0%	13.7%

Results from these small-scale experiments indicate that there are no differences in overall production among the three culture methods. The fatty acid profiles of the rotifers cultured with Microfeast-100 in combination with phytoplankton are currently being analyzed. Future work should focus on validation of these initial results at production scale. The results of those trials and the fatty acid profiles can then be used to determine the best method of culturing rotifers for use in the rearing of grouper larvae.

The size frequency distribution of the S-type rotifers produced at GADTC (Figure 9) was determined by measuring the lorica length of rotifers that were not carrying eggs, using a compound microscope equipped with an ocular micrometer. The size of the mouth of grouper larvae examined on Day 4 posthatching suggests they can physically eat rotifers less than 150 µm. This would mean that only 19% (i.e., shaded area of Figure 9) of the rotifers currently cultured are small enough for first-feeding grouper larvae. The practice of sieving rotifers through a 150 µm nytex screen has been found to be extremely wasteful and labor intensive. In addition, the screened rotifers can increase in size within six hours which would make this practice almost pointless. An alternative is to establish a population of smaller size rotifer at GADTC. A super small strain of rotifer (i.e, SS-type, average lorica length 142 µm) has reportedly been used successfully in the rearing of *E. fuscoguttatus* larvae. A starter culture was provided by Dr. Atsushi Hagiwara, Nagasaki University, Japan. Due to a misunderstanding of schedules, this particular starter culture did not survive the trip from Japan. It is recommended that further attempts be made to establish this strain of rotifers at GADTC for use in the larval rearing of grouper.

Harvest of Copepods

Copepods have been reported to be an important larval fish food if they can be cultured or harvested in sufficient numbers (Fukusho, 1991). Since the concrete raceways at GADTC that hold

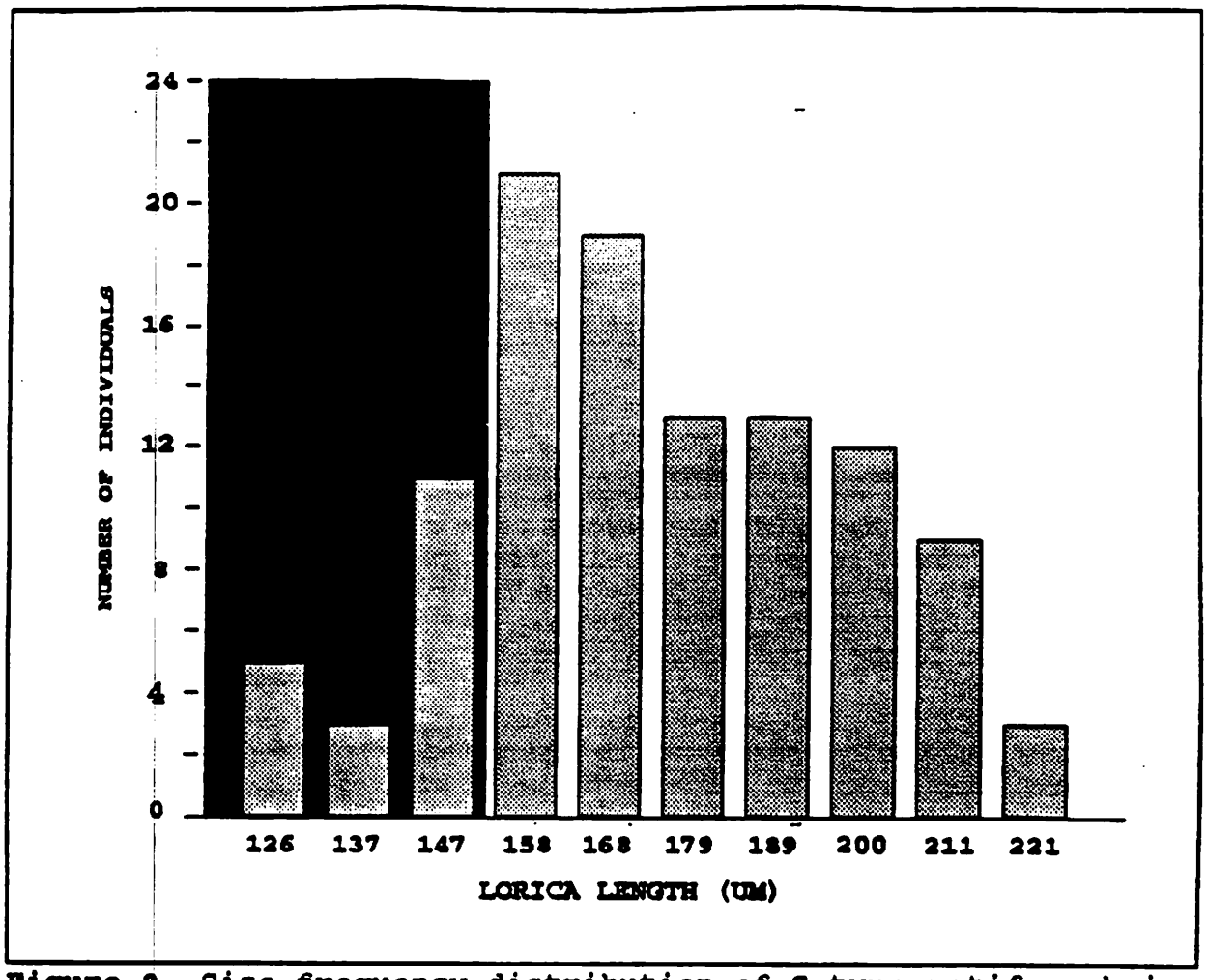


Figure 9. Size frequency distribution of S-type rotifers being cultured at GADTC. Shaded area represents size classes that are acceptable to grouper larvae.

shrimp (*Penaeus stylirostris*) broodstock were observed to have a large number of copepods, a study was done to determine their relative abundance. The harvest was conducted at night using a siphon hose to filter the raceway water though an aquarium scoop net and collect the copepods in a 60 µm mesh bag (i.e., rotifer collecting net). The collecting net was suspended in a plastic tub filled with seawater. A flashlight was placed just above the siphon hose to attract the copepods. The siphoning was conducted for one hour on four consecutive evenings. The raceway conditions were altered previous to two of the evening collection efforts. One day the tank raceway was cleaned of debris and on another day a prophylactic formalin/copper treatment for "black gill" had been added to the water. The results are summarized in Table 13. Approximately 85,000 copepods were harvested per hour of collection on the two evenings when there was no disruption to the raceway. However, the numbers of copepods that could be harvested fell

dramatically when the broodstock tanks were cleaned and/or the broodstock administered treatment. Collection of copepods was also attempted during daylight hours but resulted the harvest of very few (<1000/hour) individuals.

Table 13. Summary of harvesting copepods at night from Raceway #2 at GADTC.

Date	# Harvested/Hour	Conditions
July 20	86,111	- Normal
July 21	84,630	Normal
July 22	2,700	Raceway Bottom Siphoned
July 23	8,000	Formalin/Copper Treatment

The number of copepods harvested were significant enough to be used in the larval rearing trials for grouper, and one of the initial harvests was used to inoculate the extensive larval-rearing tank (see section Extensive Larval Rearing). Presently, an analysis of the fatty acid composition of the collected copepods is being conducted.

By harvesting the copepods as described, the younger stages of the copepods were also captured in the process. In addition, many of the adult copepods were found to be carrying eggs (Appendix 6, Plate 3), and thus are a natural source of seed for young copepods when stocked into larval-rearing tanks. The young copepods are in the 70-100 μm size range which is suitable for an initial feed for grouper larvae. A comparison of a rotifer and a copepodite is presented in Appendix 6, Plate 4.

Artemia Nauplii Production

Production of *Artemia* nauplii is constantly ongoing at GADTC for use in the rearing of shrimp postlarvae and larval catfish. An example of a newly hatched *Artemia* nauplii is presented in Appendix 6, Plate 2. All of the feed organisms were photographed at the same magnification to illustrate the size similarities and differences among them. It has been reported that *Artemia* cysts are commercially available from three major sources: San Francisco Bay, The Great Salt Lakes in Utah and Saskatchewan in Canada (Sorgeloos et al., 1991). As cysts from all three sources are characteristically deficient in the long chain polyunsaturated fatty acids, samples of newly hatched and enriched (with Protein Selco at 300 ppm) nauplii were obtained and are currently being analyzed. A comparison of the fatty acid profiles from newly hatched and *Artemia* nauplii enriched with two types of boosters are presented in Table 14 (obtained from Tamaru et al.,1993b). The two enrichment media significantly elevate the total fat content of the nauplii, in particular, both C20:5n-3 and C22:6n-3 (i.e., EPA and DHA, respectively) were significantly elevated.

Table 14. Fatty acid profiles of newly hatched and enriched *Artemia* nauplii. Values are the means from triplicate determinations and are expressed in terms of mg/100 mg dry weight. The data was obtained from Tamaru et al., 1993b.

Fatty Acid	Newly Hatched *Artemia* Nauplii	Enriched *Artemia* (Menhaden Oil) 300 ppm	Enriched *Artemia* (Super Selco) 100 ppm
C14	0.06	0.36	1.13
C16	0.82	1.77	1.68
C16:1n-7	0.32	0.75	0.40
C18	0.53	0.77	0.80
C18:1n-9	1.52	2.38	2.81
C18:2n-6	0.49	0.64	0.68
C18:3n-3	1.94	2.57	2.58
C18:4n-3	0.28	0.47	0.18
C20:1n-9	0.05	0.12	0.23
C20:4n-6	0.17	0.20	0.14
C20:5n-3	0.36	1.02	1.28
C22:1n-11	n.d.	0.02	0.06
C22:6n-3	n.d.	0.50	0.69
TOTAL	6.55	11.55	11.66

n.d. = not detected

This data and the report by Lin et al., (1991) suggest that *Artemia* nauplii used in the rearing of grouper larvae should be enriched. Therefore, the staff at GADTC should become familiar with and include procedures for boosting the fatty acids of *Artemia* nauplii as part of their daily activities.

Penaeus stylirostris nauplii

One of the current activities at GADTC is the hatchery production of *P. stylirostris* juveniles for the shrimp industry in Guam. Nauplii production (i.e., 100-300 x 10^3/day) from mated females occurs daily but rearing is not conducted for all spawns due to the lack of rearing space. Likewise, the nauplii are only reared in response to demand. In short, a potentially large number of nauplii that are equivalent in size to that of *Artemia* nauplii are being disposed of. Samples of nauplii were obtained and the fatty acid analyses are in progress. The nutritional analysis is being conducted to evaluate the potential use of the excess nauplii as a feed for grouper larvae.

Sea Urchin, *Tripneustes gratilla*

Sea urchin eggs have long been used in the study of embryonic development because of their availability and the ease by which spawning can be manipulated. Sea urchin larvae can also serve as an alternative initial feed to oyster trochophores because of their size (70-100 μm). During this consultancy, several locations around Guam were surveyed to determine areas where this species can be obtained. Two locations on the west shores of Guam (i.e., Tumon Bay and Asan Point) were devoid of this species. However, this species of sea urchin was found in the holding facilities at the University of Guam Marine Laboratory on the east coast of the island. Interviews with staff indicate an abundance of this species just offshore of the laboratory.

A procedural guide to the induction of spawning this species is provided in Appendix 7, courtesy of Dr. Samuel Haley, Department of Zoology, University of Hawaii at Manoa. It is recommended that a broodstock of *T. gratilla* be established at GADTC and the reproductive season be defined both in captivity and in the wild.

EFFECT OF LIGHT ON NEWLY HATCHED LARVAE

It has been reported that grouper larvae are extremely sensitive to light, particularly from the time of hatching to first feeding (Lin et al., 1991). One rearing trial of *E. microdon* larvae during the first phase of the consultancy was conducted in 3000-liter fiberglass tanks situated in a back room of the hatchery at GADTC. Although this area is the darkest area in the hatchery, total mortality was observed prior to Day 3 posthatching (i.e., prior to initial feeding). A similar observation was made at MMDC where the newly hatched larvae were held in glass aquaria.

These observations prompted an experiment during the second phase of the consultancy. At the MMDC facility, spawned *E. microdon* eggs were stocked at a density of 100-150 eggs/l and allowed to hatch in two glass aquaria equipped with continuous aeration. One aquarium was covered with black plastic sheet while the other was exposed to ambient light conditions. Larval survival was determined volumetrically and survival was recorded at various intervals (Figure 10). As observed previously, the newly hatched larvae of *E. microdon* appeared to be sensitive to light and covering of the larval-rearing tanks during the first three days posthatching must be undertaken with all future larval rearing trials to ensure survival.

FIRST FEEDING EXPERIMENT

Small-scale (15-liter) larval rearing experiments were conducted during both phases of the consultancy to evaluate the use of available live food organisms for the initial feeding of grouper larvae. During the first phase of the consultancy, fifteen 15-liter plastic tanks were stocked with 300 larvae/tank (i.e., 20

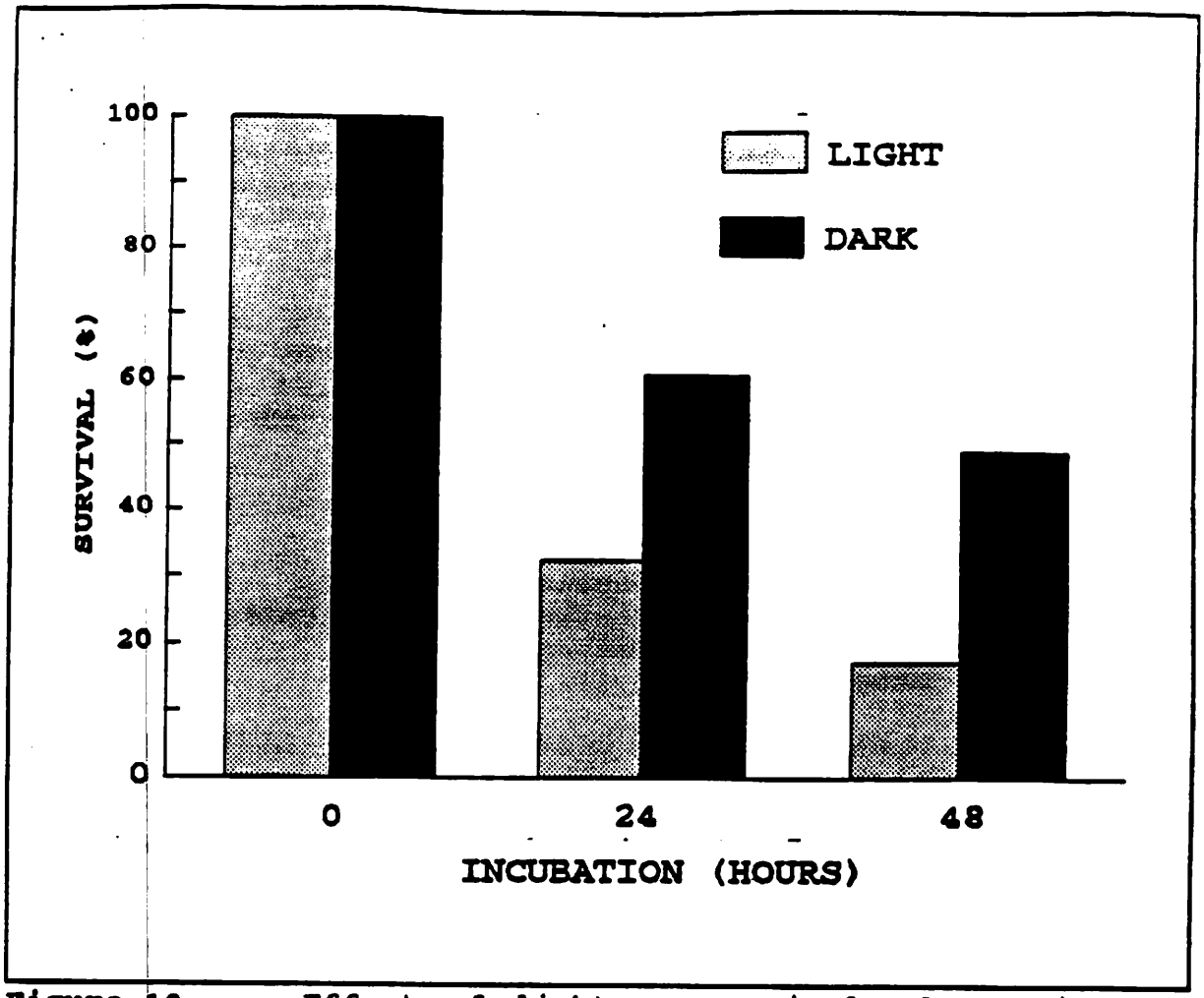


Figure 10. Effect of light on survival of *E. microdon* larvae.

larvae/l) at a salinity of 34 ppt. Each tank was provided aeration via a single air stone and stocked with phytoplankton at a density of 300-500 x 10^3 cells/ml. Each day, 25% of the volume of water was siphoned from each tank and replaced with phytoplankton. All tanks were covered with a shade cloth throughout the course of the experiment. Five different feeding treatments were conducted and triplicated. The experiment was carried out for seven days posthatching. The treatments were:

TRIAL 1

Treatment #1 Oyster trochophores (20/ml)
Treatment #2 Oyster trochophores (10/ml) + Rotifers (10/ml)
Treatment #3 Rotifers (40/ml)
Treatment #4 Rotifers (40/ml) + ML-100 Artificial feed
Treatment #5 No feed (Control)

High mortalities were observed between Day 2 and Day 3 posthatching. By the end of the experiment only three larvae survived. Two larvae were found in a tank receiving Treatment #2 and one larvae remained in a tank receiving Treatment #1. Because of the large number of mortalities that occurred prior to first feeding, the results are inconclusive. However, that surviving larvae were present in treatments containing oyster trochophores provides indication of the importance of this product as an initial feed.

The rearing experiment during the second phase of the consultancy was conducted using the same rearing tanks and under the same conditions except that the exterior of each tank was painted black to prevent exposure to light. The experiment design was as follows:

TRIAL 2

Treatment #1 Oyster trochophores only (20/ml)
Treatment #2 Oyster trochophores (10/ml) + Rotifers (30/ml)
Treatment #3 Rotifers only (40/ml)
Treatment #4 No feed (Control)

The second rearing trial also sustained high mortalities during the initial three days posthatching and the experiment was terminated on Day 5. The experiment was terminated since the larvae in the control tanks (i.e., no feed) died and the results of the feed could thus be evaluated (Figure 11). Overall survival was very poor (i.e., <1.0%) and no significant differences could be detected among treatments (analysis of variance). Although there is no statistical validity to the results, once again the highest survival was observed in treatments employing oyster trochophores. Future work should focus on varying the rotifer/trochophore ratios at different larval densities to obtain the optimal ratios of feed to larvae. Small-scale trials should be covered with a black plastic sheet during the first three days posthatching.

It should be emphasized that these trials were conducted at laboratory scale. Extrapolation of the results to commercial scale indicate that the use of oyster trochophores would not be economically feasible, thus warranting a review of alternative feeds of similar size (e.g., sea urchin, trochus, giant clam, and mussel larvae) during future research activities.

INTENSIVE LARVAL REARING

Intensive larval-rearing trials were conducted in 3000-liter fiberglass tanks during both phases of the consultancy. During the first phase, two tanks were stocked with spawned eggs from *E. microdon* and two tanks were stocked with eggs from *E. fuscoguttatus* at a density of 20 eggs/l. The salinity of the water ranged between 32 and 34 ppt and moderate aeration was provided to each tank. The tanks were filled with only 1000 liters of water because

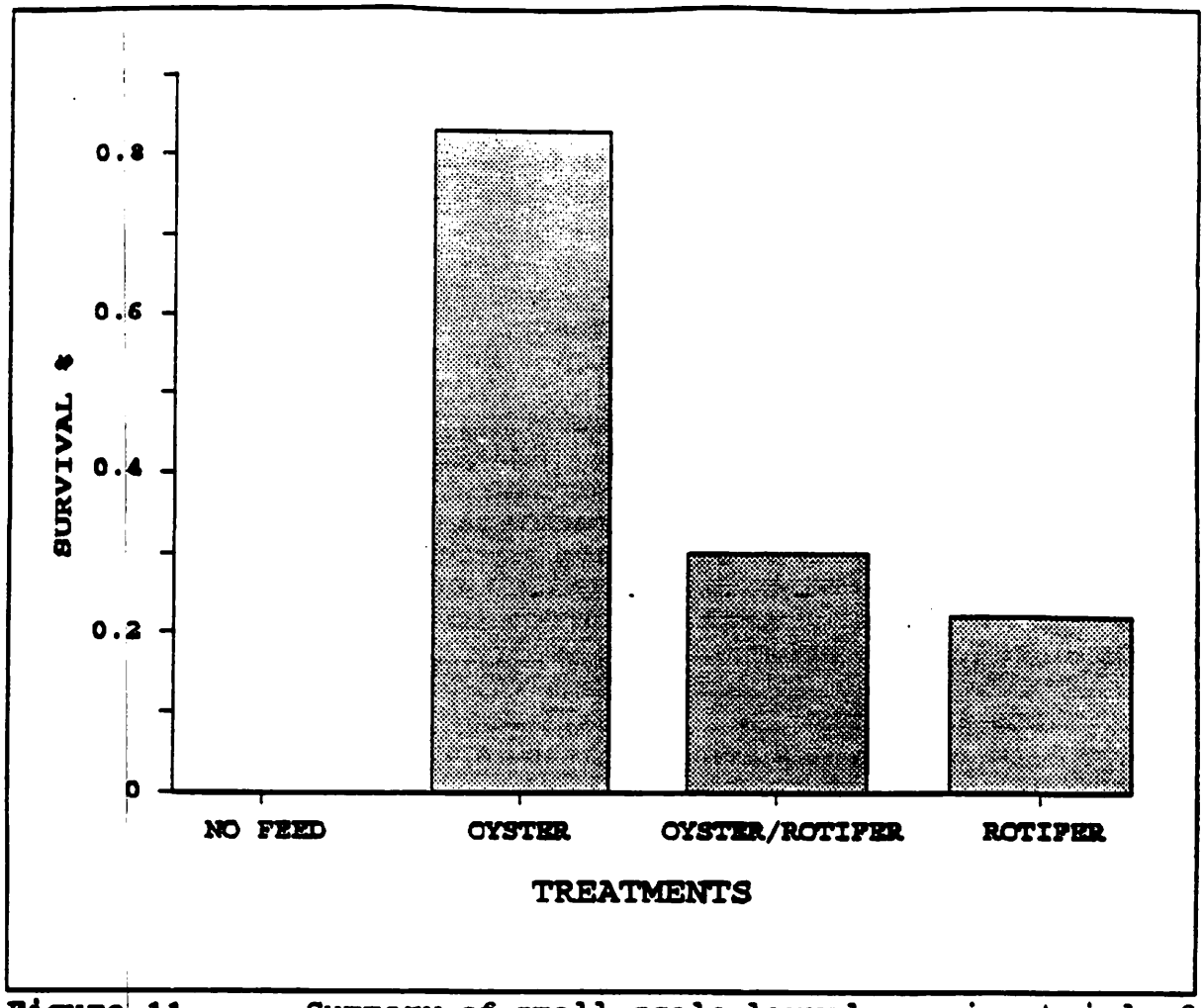


Figure 11. Summary of small-scale larval rearing trial of *E. microdon*.

of the rationing of the oyster trochophores. The hatching percentage was estimated to be 20%, resulting in an initial larval density of 4 larvae/l. All tanks were stocked with oyster trochophores (10/ml), S-type rotifers (30-40/ml), and phytoplankton at a density of 300-500 x 10^3 cells/ml from Day 2 posthatching. Initially, only the tanks containing eggs from *E. fuscoguttatus* were provided with shade cloth. The feeding regimen was to remain the same until Day 12 posthatching at which time *Artemia* nauplii would be introduced at an initial density of 0.01/ml and increased depending upon the amount of consumption by the larvae.

Major mortality of *E. microdon* larvae was observed by Day 3 posthatching and the rearing trial was terminated. The mortality occurred prior to the feeding of larvae and the lack of a shade cloth cover was presumed to be the cause.

The initial larval survival of *E. fuscoguttatus* remained relatively high over the first five days posthatching. Major mortality occurred on Day 6 posthatching and the rearing trial was

terminated on Day 7. The lack of oyster trochophores was presumed to be the major cause in this case. Project funding allowed for enough trochophores to feed the larvae only until Day 5 posthatching.

During the second phase of the consultancy, four rearing tanks were stocked with eggs from *E. microdon* at a density of 40 eggs/l. The tanks were again filled at a low level of 1000 liters to ration the oyster trochophores and initial feeding was delayed until Day 3 posthatching. The four tanks were divided into two separate treatments (i.e., two received oyster trochophores and rotifers at densities of 10 trochophores/ml:30 rotifers/ml and two received rotifers only at 40 rotifers/ml). All tanks were covered with shade cloth and black plastic sheeting continuously during the initial three days posthatching. Addition of phytoplankton and daily maintenance was carried out the same as described in the first rearing trial. Larval survival in all tanks was much improved during the first five days posthatching in comparison to the first trial, where total mortality occurred by Day 3. Heavy mortality in the second trial occurred on Day 6 and the experiment was terminated. The preliminary results indicate that the feeding ratio of trochophores:rotifers needs further investigation. Based on the trends observed in the small-scale rearing trials, future trials should employ a higher initial stocking density of oyster trochophores (i.e., at recommended density of 20 trochophores/ml:20 rotifers/ml) and initial feeding should begin on Day 2 posthatching.

EXTENSIVE LARVAL REARING

One extensive larval-rearing trial was conducted in a 20,000-liter fiberglass tank during the initial phase of the consultancy. Tank preparation began eight days prior to the stocking of *E. fuscoguttatus* eggs. Phytoplankton was stocked into the tank at an initial density of 1-5 x 10^6 cells/ml and allowed to grow. On the second day after the introduction of phytoplankton, 70,000 copepods collected from Raceway #2 were stocked into the tank. Rotifers were then stocked into the tank on the fourth day after introduction of the phytoplankton. Rotifers were added at an initial density of 5 rotifers/ml and allowed to grow over the next three days to attain a density of 15-20 rotifers/ml when the eggs were stocked. Egg stocking density was 2 eggs/l. Although the rearing tank was covered with a shade cloth, heavy rains lowered the salinity to 27 ppt at the time of stocking. By first feeding, rotifer densities remained at 20 rotifers/ml and estimates of copepod densities indicated that their population had at least doubled during the same time period. On the last day of the first phase of the consultancy (Day 10 posthatching), several hundred 10-day-old grouper larvae could be seen in the tank, apparently and growing in size. GADTC staff attempted to rear the larvae through to juvenile stages (i.e., 25-30 days posthatching). Estimating survival in this tank was very difficult but using the 20% hatching

rate observed in the intensive rearing tanks, an estimated 8000 larvae would have been present at hatching. A 10% survival would indicate approximately 800 larvae, which is a close approximation of the amounts present on Day 10 posthatching. Larvae were detected until Day 15 posthatching, after which no larvae could be found. Upon arrival at GADTC for the second phase of the consultancy, the consultant found a large number of medusae in the larval-rearing tank at a density of 5-10/l. The presence of medusae is the probable cause of mortality in this trial as they are carnivorous and feed on larvae as well as live feed organisms. Interviews with the staff at GADTC indicate the occurrence of medusae during other rearing trials at GADTC and this appears to be an ongoing problem. An examination of the entire system, and especially the main seawater source is warranted to determine the origin of the medusae. One known location (Bauerlein, pers. comm.) of the medusae is in the shrimp broodstock raceway that was the source of copepods collected during the project. Unfortunately, this fact was not known to the consultant prior to the discovery of medusae in the extensive tank. It is recommended that future rearing trials employ filters (5-10 μm) on <u>all</u> incoming water sources to prevent this occurrence. Formalin treatments to rid medusae in existing stock tanks were found fatal to the live feeds and potentially to the larvae, as described in the following section.

Prior to the discovery of medusae, it is interesting to note that the larvae in this extensive rearing trial did not receive any oyster trochophores, yet it remained the most successful rearing trial. An examination of the copepods in the tank and those that were collected from Raceway #2 revealed the presence of a large number of copepodites that were similar in size (i.e., 70-100 μm) to oyster trochophores. It is presumed that the larvae were feeding on these organisms. A second observation is that the larvae were doing well at a reduced salinity. Investigations of the effects of salinity on larval growth and survival vary with the species. There are reportedly no effects of salinity on larval growth and survival for striped mullet larvae (Murashige et al., 1991). In contrast, larval rearing of milkfish is optimal at salinities between 20 and 25 ppt (unpublished data). Future work should focus on the optimal salinity for the larval rearing of grouper.

During the second phase of the consultancy, another extensive larval-rearing trial was conducted in a different 20,000-liter tank. Tank preparation was conducted as described previously. However, strong rains reduced the salinity to 25 ppt when spawned eggs of *E. microdon* were stocked at a density of 20 eggs/l. No hatching was observed during this rearing trial. It is recommended that future extensive rearing trials be undertaken in tanks covered with a shade cloth attached as close to the water level as possible. In addition, the entire tank area must be covered with a waterproof tarp to keep out the rain. It is crucial that the coverings (i.e., tarp and shade cloth) be securely fastened to keep

the low light environment constant as well as to withstand the high winds and rain that occur in Guam.

A third extensive larval-rearing trial was attempted using a 100 m^2 concrete pond stocked at a density of 2 eggs/l. The pond was prepared in similar fashion to the extensive larval-rearing tank. However, because of its size, no waterproof covering could be affixed to this facility and a decrease in salinity occurred due to rain. This trial also resulted in a poor hatch, as no larvae could be seen in the pond after stocking.

An additional problem with the larger extensive system was the establishment of phytoplankton. The water in the tank needs to be nutrified and careful monitoring of the phytoplankton must be carried out. Variability in the available sunlight will lead to variations in cell density and ultimate changes in pH. Future work should focus on gaining experience in managing pond conditions and food organisms. Critical operating parameters that must be maintained are a salinity of 32-35 ppt, algal densities of 300-500 x 10^3 cells/ml, rotifers at 5-10/ml, and the presence of copepods.

EFFECT OF FORMALIN ON SURVIVAL OF LIVE FEEDS

The presence of the medusae (See Appendix 6, Plate 5) in a larval rearing trial is an extremely serious condition as there is no known remedy to this situation without mortality to the larvae. One treatment known by the consultant to disinfect eggs was evaluated for effectiveness in eliminating the medusae found in the extensive rearing tank at GADTC. The treatment consists of immersing the eggs into a 100 ppm formalin bath for one hour. Spawned eggs from a variety of species are not effected by concentrations as high as 1000 ppm (unpublished data), but it remained to be seen whether the live feed organisms could tolerate such a treatment. Medusae, rotifers, copepods, and *Artemia* nauplii were placed into 1-liter beakers and subjected to various concentrations of formalin in seawater. After one hour the number of surviving individuals were quantified either volumetrically or by direct count. The results (Figure 12) indicate that a dosage of 50 ppm is fatal to the medusae. However, it is apparent that rotifers and copepods are also as sensitive to the treatment and thus the treatment cannot be used in the event that an outbreak of the medusae occur during the early stages of a larval rearing trial. *Artemia* nauplii were found to be unaffected by formalin at concentrations as high as 1000 ppm. The use of formalin during the latter stages of a rearing trial, when this feed is used exclusively therefore does warrant further investigation. The sensitivity of the larvae to formalin at various stages of development would provide an indication of whether this treatment can be used at all.

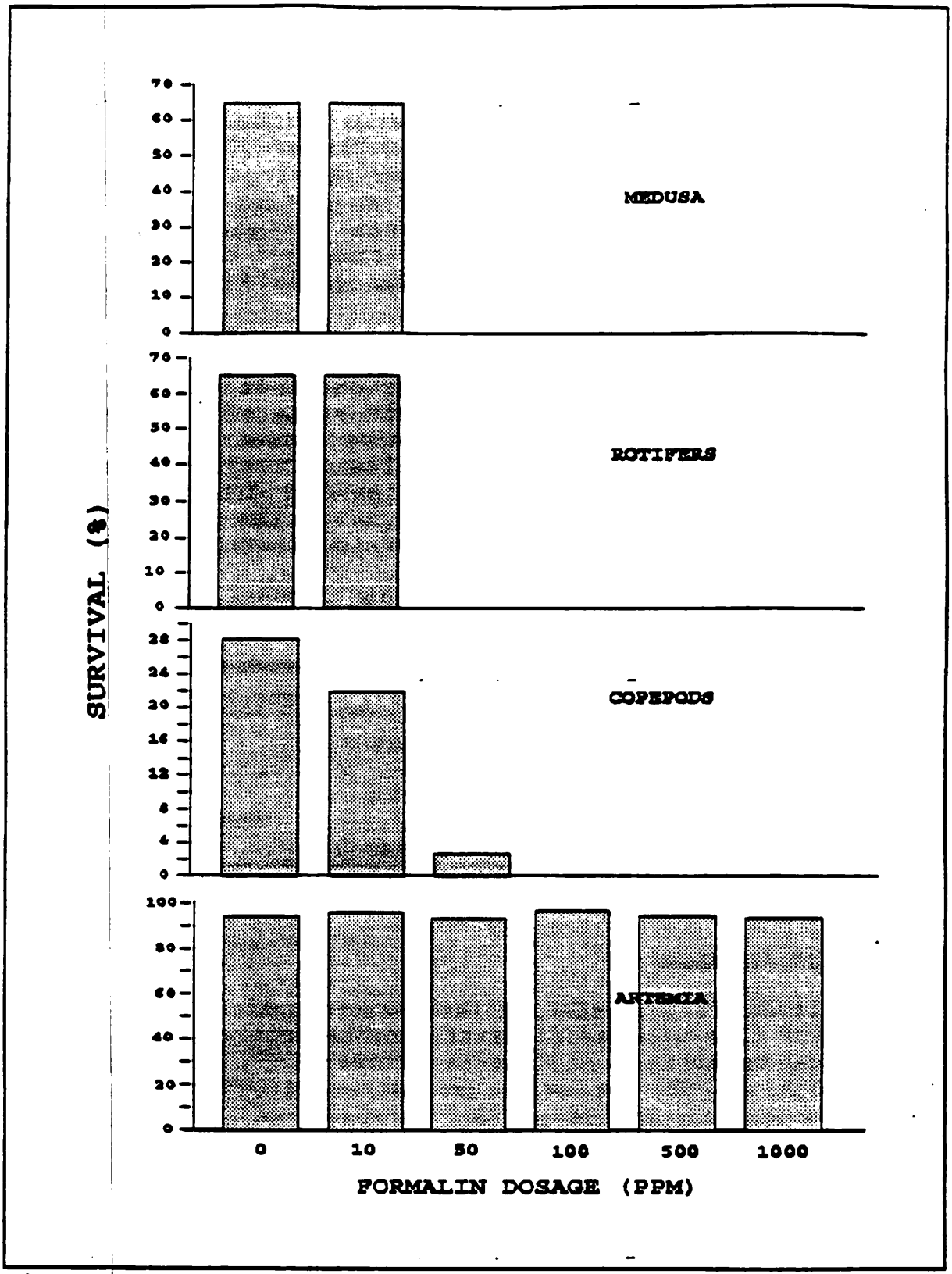


Figure 12. Effect of various concentrations of formalin on live feeds and medusae.

OTHER GROUPER SPECIES

One of the tasks of this project was to assess the reproductive seasons of various species of groupers. Several species under consideration for culture in addition to *E. microdon* and *E. fuscoguttatus* were collected. Pictures of *E. microdon*, *E. fuscoguttatus* and some of the other groupers species encountered during the consultancy are presented in Appendix 8, Plates 1-6. When possible, biological data from gonadal biopsies and length and weight measurements were obtained. A summary of data obtained for *Plectropomus areolatus* and *Cephalopholis miniata* follows.

Plectropomus areolatus

Five *P. areolatus* individuals were captured by hook and line on July 13, 1993, and examined for their state of maturity (Table 15). Previous data regarding the spawning season for this species (FitzGerald et al., 1991) and the results of the gonadal biopsies indicate that the peak in the spawning season had already passed by the initial phase of this consultancy. After the individuals were examined they were returned to the spawning channel.

Table 15. Summary of data obtained by gonadal biopsies of *P. areolatus* captured on July 13, 1993.

Number	Body Weight (kg)	Sex	State of Maturity
1	2.42	unknown	-
2	2.78	male	cann. sperm
3	1.66	unknown	-
4	2.52	male	cann. sperm
5	1.42	female	pv

cann. sperm = cannulated sperm
pv = previtellogenic oocytes

Cephalopholis miniata

Individuals of this species were also caught by hook and line. The habitat of this species differed from the other groupers caught during the consultancy. Whereas *E. microdon* and *E. fuscoguttatus* were caught in the channel areas and at relatively deep depths, *C. miniata* were caught inside of the barrier reef and appeared to inhabit solitary outcroppings of coral. As many as 3-10 individuals were obtained from a single location. Attempts were made to capture individuals of this species in July and August. A summary of the state of maturity of the captured fish is presented in Table 16. Gonadal maturation was assessed from individuals that had died during transport to the laboratory by excision of their gonads and a wet mount prepared from a small section of excised gonad. The wet mounts were then viewed under a compound

microscope. Individuals scored as negative (Table 16) were alive and biopsied using a cannula but resulted in no gonadal sample being obtained. Three stages of maturity, (i.e., mature testes, transitional male, immature ovary) are presented in photomicrographs of wet mounts presented in Appendix 9, Plates 1-3.

Sperm from the male caught in July was activated when exposed to seawater. Females were found to range in maturity from possessing only previtellogenic oocytes, to being fully gravid with a few ovulated eggs. The presence of mature males, and females at various stages of maturity would indicate the existence of some reproductive activity during the months of July and August.

Table 16. Summary of gonadal biopsies obtained from *C. miniata*.

Number	Date	Total Length (cm)	Body Weight (g)	Sex	State of Maturity
1	7/17/93	20.5	183	female	pv + vit
2	7/17/93	26.2	452	male/female	transitional
3	7/17/93	28.5	516	male	cann. sperm
4	8/14/93	23.0	175	female	vit
5	8/14/93	21.5	122	female	vit
6	8/14/83	23.0	181	female	pv + vit
7	8/14/93	23.0	151	female	pv
8	8/14/93	28.6	330	male	cann. sperm
9	8/14/93	34.0	540	male	cann. sperm
10	8/14/83	25.5	301	male	cann. sperm
11	8/14/93	28.2	435	male	cann. sperm
12	8/14/93	25.1	277	male	cann. sperm
13	8/14/93	30.5	470	negative	n.a.
14	8/14/93	30.5	320	negative	n.a.
15	8/14/83	27.9	370	negative	n.a.
16	8/14/93	32.1	490	negative	n.a.

cann. sperm = cannulated sperm
pv = previtellogenic oocytes
vit = vitellogenic oocytes
transitional = individual undergoing transition in sex
n.a. = not available

The length *versus* weight relationship of the *C. miniata* individuals listed in Table 16 is summarized in Figure 13. The data was subjected to regression analysis and the following linear model was found to provide the best fit of the data:

$$BW = 31.33 * (TL) - 506.16$$
$$r^2 = 0.76$$
$$P < 0.001$$

The sex of the individuals is also presented in the graph and while all of the smaller individuals were females, the larger

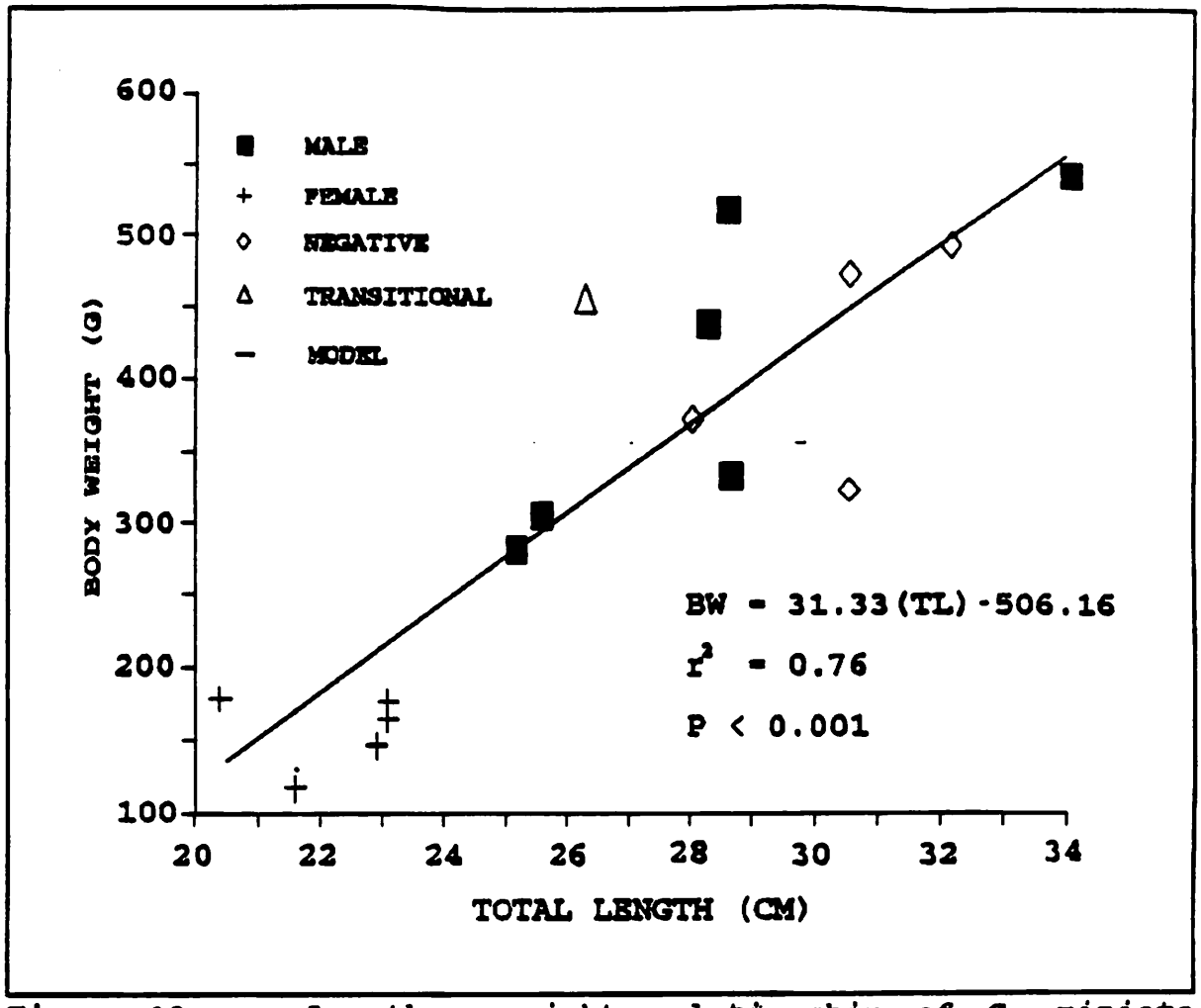


Figure 13. Length x weight relationship of *C. miniata* captured in July and August 1993.

individuals were males or of undetermined sex. From the distribution of the sexes, it would appear that the majority of individuals scored as negative were males. One individual was of a transitional sex and marks the size at which transition from female to male occurs.

A fortunate occurrence was the capture of two females during August that were fully gravid. A comparison of oocyte size frequency distribution is shown in Figure 14. One female possessed a few oocytes that had clearly ovulated, indicating that she had also spawned recently. The other female that did not have any ovulated oocytes, however, also possessed a larger number of oocytes >460 μm that might indicate the oocyte diameter most receptive to hormonal stimulation of final maturation and spawning.

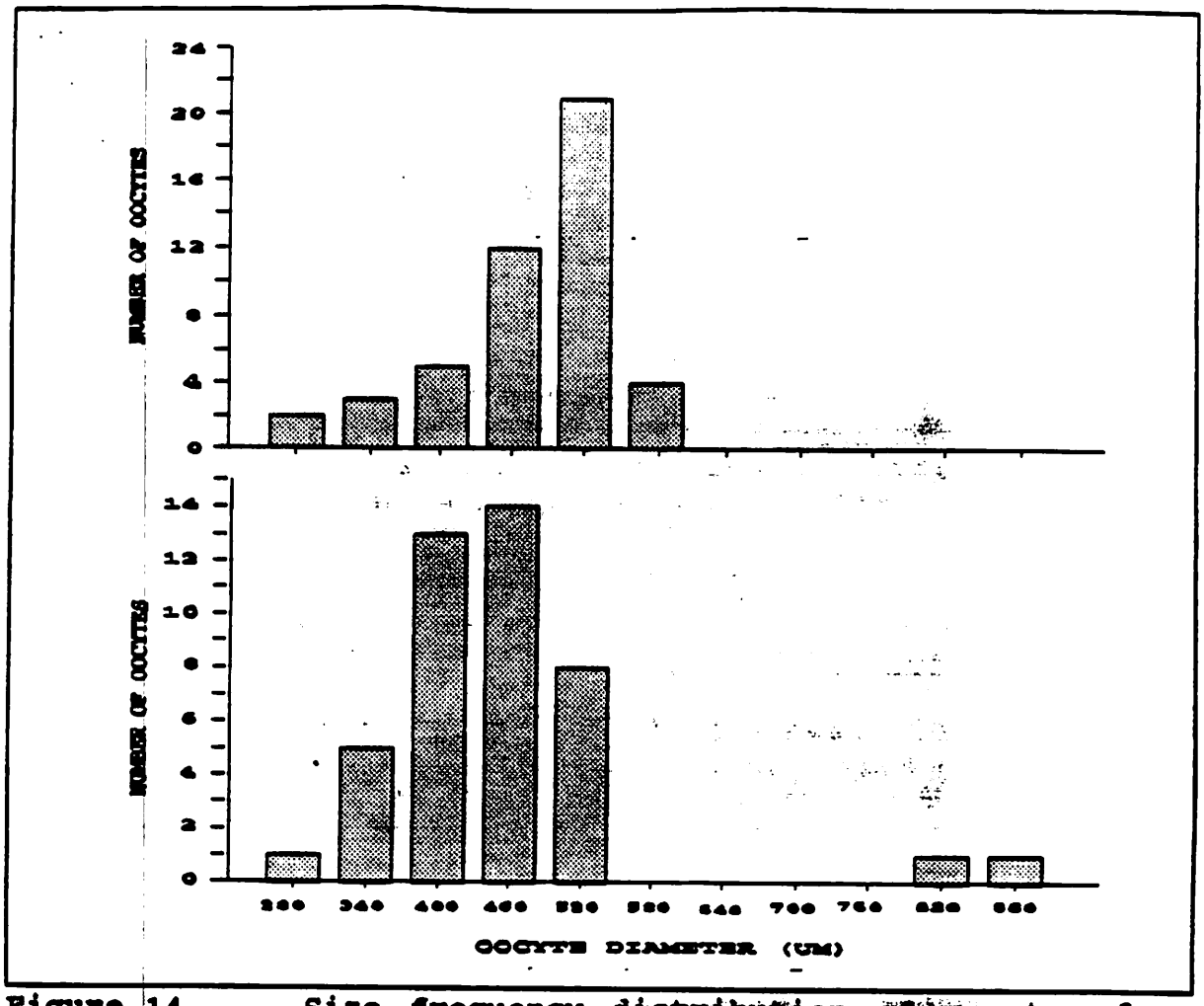


Figure 14. Size frequency distribution of oocytes from two female *C. miniata* caught on August 16, 1993.

TRANSPORT OF ADULT GROUPERS TO GADTC

E. microdon

During the first phase of the consultancy, a simulated transport experiment was conducted with two adult *E. microdon* males. The individuals, 0.91 and 0.66 kg in body weight, were placed in separate 30-gallon glass aquariums with water temperatures gradually lowered to 25 and 22°C, respectively. At these temperatures the fish were easy to handle and did not thrash when placed into plastic bags (3-ml in thickness) filled with 12 liters of seawater. The bags were filled with oxygen, sealed with rubber bands and placed in styrofoam coolers containing a small amount of ice placed in the bottom. The duration of the experiment was 12 hours, or the actual transport time if the shipment was to take place by air freight. At the end of the experiment, the fish

were removed from the bag and allowed to acclimate in a 1000-liter tank equipped with running seawater and continuous aeration. Water temperatures in the transport bags dropped to 23.5 and 19°C, respectively, by the end of the experiment. No mortalities occurred.

During the second phase of the consultancy, six adult individuals (3 males and 3 females) were transported successfully to GADTC. However, one female died after the first injection of hormone in an attempt to induce spawning. Another female and male died when a female tried to eat a male of comparable size (See Appendix 10, Page 10). They were found locked together at the bottom of the tank. Twelve-inch PVC fittings were placed into the holding tanks to provide hiding places for the groupers. All fish received antibiotic treatments in response to fin rot most likely due to trauma received during capture and transport activities. The antibiotic treatment consisted of Prefuran (5 ppm for one hour) repeated over a five-day period. At last observation on August 24, 1993, all individuals were eating and appeared healthy.

E. fuscoguttatus

Three adult female *E. fuscoguttatus* individuals, 2.69, 3.21, and 3.35 kg in body weight, respectively were shipped from Palau to Guam. The preparation was the same as that in the transport experiment using *E. microdon*. When the shipping boxes arrived in Guam, the boxes were opened at the airport. Water temperatures were exceeding low, (i.e., ranging from 9-13°C) and the fish were massaged to regain mobility. One individual died within hours and the remaining two individuals died two days later. Although the experiment ended in mortality, a protocol was established. With modifications in the placement and amount of ice, adult *E. fuscoguttatus* groupers can be shipped from Palau to establish a broodstock at GADTC.

Cephalopholis miniata

To establish a broodstock of this species at GADTC, 44 individuals captured by hook and line were shipped using the same methods as described. Upon arrival at GADTC these individuals received Prefuran treatments and mortalities (n=4) were due only to infections that resulted from capture and transport activities. At last observation all remaining fish were eating and healthy.

SUMMARY AND RECOMMENDATIONS

Although all the objectives and then some were accomplished during this consultancy, a hatchery technology for the selected grouper species has yet to be fully established at GADTC and validated at commercial scale. One major problem that must be addressed is the presence of medusae in the rearing tanks at GADTC. The source(s) need to be located and corrective action taken in

order to minimize the chances of medusae entering into the larval rearing system in the future.

The following section represents recommendations for areas that require additional work in order to establish a protocol for the artificial propagation of groupers studied during this consultancy. They are:

Reproduction

- Establish captive broodstock of groupers targeted for domestication
- Define the spawning season of selected groupers both from the wild and in captivity based upon gonadal maturity
- For each species, determine the size at first maturity and size at which the transition from female to male occurs
- Determine the stage of ovarian maturation most receptive to hormonal induction of spawning
- Establish a broodstock that spawns naturally (i.e., with no hormone treatment)
- Determine the minimum effective dosage of HCG for induction of final maturation and spawning
- Determine the effectiveness of alternative hormones (i.e., carp pituitary homogenate, LHRH-a) for inducing final maturation and spawning
- Determine the effectiveness of 17-MT on induction of testicular maturation in captive broodstock
- Determine the effectiveness of 17-MT on induction of sex reversal of captive broodstock
- Determine the effects of chronic LHRH-a treatment on ovarian maturation
- Determine the optimal temperature:salinity combinations for hatching of grouper eggs

Larval Rearing

- Determine the light intensity of which newly hatched grouper larvae are tolerant
- Determine optimal initial larval:feed (i.e., oyster trochophores, rotifers) densities at first feeding

- Establish a population of sea urchins at GADTC and determine the availability of sea urchin larvae throughout the year

- Determine the suitability of alternative initial live feeds (i.e., sea urchin, trochus, mussel, giant clam larvae)

- Establish and mass culture the smaller strain (i.e, SS-type rotifer) at GADTC for use in larval rearing of groupers

- Determine feeding preference of grouper larvae at various stages of development

- Determine the optimal salinity for larval culture

- Determine the effects of enriched *versus* nonenriched *Artemia* nauplii on larval growth, survival and stress resistance

- Determine the effects of different levels of C20:5n-3 and C22:6n-3 on larval survival, growth and stress resistance

- Incorporate the results of the experiments into a larval rearing protocol and validate at commercial scale

- Adapt the larval rearing protocol to other grouper species

- Gain experience in manipulating extensive larval systems to remain in the critical operating parameters (i.e., salinity 32-35 ppt, phytoplankton densities of 300-500 x 10^3 cells/ml, rotifer densities of 10-20/ml, and the presence of copepods

- Determine the optimal larval stocking density and food density in extensive (i.e., 20,000-l) rearing systems

LITERATURE CITED

Anonymous. 1989. Fishery statistics bulletin for South China Sea area. 1987. Southeast Asian Fisheries Development Center, Thailand. 162 pp.

Chao, N.-H, H.-P. Chen and I.-C. Liao. 1975. Study on cryogenic preservation of grey mullet sperm. Aquaculture, 5:389-406.

Chao, T.M., L.-C., Lim and L.T. Khoo. 1991. Studies on the breeding of brown marbled grouper *Epinephelus fuscoguttatus* (Forsskal) in Singapore. Paper presented at: Finfish Hatchery in Asia '91 Conference, Tungkang, Taiwan, December 17-19, 1991. 24 pp.

FitzGerald, W. Jr., M. Bauerlein and T. Behrenfeld. 1991. Preliminary spawning and culture of groupers in Palau and Guam. Final Report, Pacific Aquaculture Association. Honolulu, Hawaii USA, pp. 1-53.

Fukusho, K. Review of the research status of zooplankton production in Japan. In: W. Fulks and K.L. Main (Eds.), Rotifer and Microalgae Culture Systems, Proceedings of U.S.-Asia Workshop. Oceanic Institute, Hawaii, U.S.A. pp. 55-60.

Johannes, R.E. 1981. Words of the Lagoon: Fishing and Marine Lore in the Palau District of Micronesia. University of California Press, Berkeley CA. 245 pp.

Kitalong, A. and E. Oiterong. 1991. Review of the grouper fishery for two predominant species: *Epinephelus fuscoguttatus* and *Plectropomus leopardus*. Marine Resources Division, Koror, Palau. 10 pp.

Kohno, H., M. Durray and P. Sunyoto. 1990. A field guide to groupers of Southeast Asia.24 Pusat Penelitian dan Pengembangan PHP/KAN/PT.No. 14/1990 Jakarta, 26 pp.

Lee, C.-S., C.S. Tamaru, C.D. Kelley and J.E. Banno. 1986. Induced spawning of milkfish, *Chanos chanos*, by a single application of LHRH-analogue. Aquaculture, 58:87-98.

Lee, C.-S., C. S. Tamaru, C.D. Kelley, G.T. Miyamoto and A. Moriwake. 1992a. The minimum effective dosage of 17 α-methyltestosterone for induction of testicular maturation in striped mullet, *Mugil cephalus* L. Aquaculture, 104:183-191.

Lee. C.-S., C.S. Tamaru, C.D. Kelley, A. Moriwake and G.T. Miyamoto. 1992b. The effect of salinity on the induction of spawning and fertilization in the striped mullet, Mugil cephalus. Aquaculture, 102:289-296.

Lubzens, E. 1987. Raising rotifers for use in aquaculture. Hydrobiologia, 147:245-255.

Murashige, R., P. Bass, L. Wallace, A. Molnar, B. Eastham, V. Sato, C. S. Tamaru and C.-S. Lee. 1991. The effect of salinity on the survival and growth of striped mullet, Mugil cephalus, larvae in the hatchery. Aquaculture, 96:249-254.

Morisawa, M. 1985. Initiation of sperm motility at spawning in teleosts. Zoological Science, 2:605-615.

Shehadeh, Z.H., C.-M. Kuo and K. Milisen. 1973. Validation of an *in vivo* method for monitoring ovarian development in the grey mullet (*Mugil cephalus*). J. Fish. Biol., 5:489-496.

Sorgeloos, P., P. Lavens, Ph. Leger and W. Tackaert. State of the art in larviculture of fish and shellfish. 1991a. In: P. Lavens, P. Sorgeloos, E. Jaspers, and F. Ollevier (Eds.). Larvi '91 - Fish & Crustacean Larviculture Symposium, European Aquaculture Society, Special Publication No. 15, Gent, Belgium. pp. 3-5.

Sorgeloos, P. P. Lavens, Ph. Leger and W. Tackaert. 1991b. The use of *Artemia* in marine fish culture. Paper presented at : Finfish Hatchery in Asia '91 Conference, Tungkang, Taiwan. Dec. 17-19, 1991. 12 pp.

Tamaru, C.S., C.-S. Lee and H. Ako. 1991a. Improving the larval rearing of striped mullet, (Mugil cephalus) by manipulating quantity and quality of the rotifer, Brachionus plicatilis. In: W. Fulks and K.L. Main (Eds.), Rotifer and microalgae culture systems. Proceedings of a U.S.- Asia workshop. The Oceanic Institute, Hawaii, U.S.A. pp.89-103.

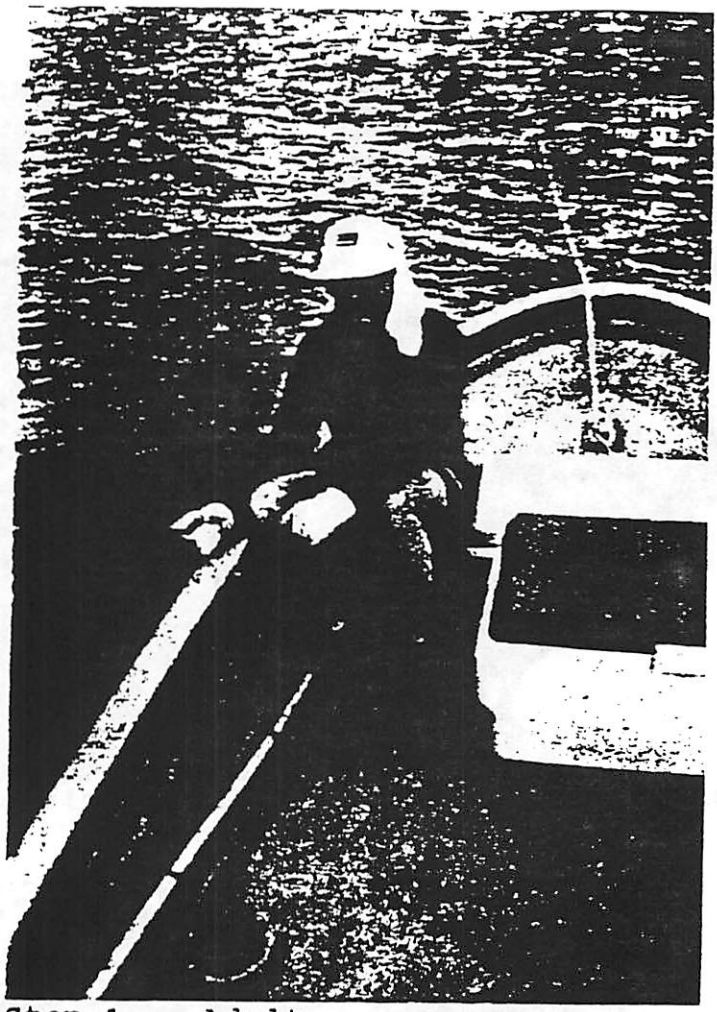
Tamaru, C.S., C.D. Kelley, C.-S. Lee, K. Aida, I. Hanyu and F. Goetz. 1991b. Steroid profiles during maturation and induced spawning of the striped mullet, *Mugil cephalus* L.. Aquaculture, 95:149-168.

Tamaru, C.S., F. Cholik, J. C.-M., Kuo and W. FitzGerald, Jr. 1993a. Status on the culture of milkfish (*Chanos chanos*), striped mullet (*Mugil cephalus*) and grouper (*Epinephelus* sp.). Paper presented at World Aquaculture '93, Torremolinos, Spain, May 26-28, 1993. European Aquaculture Society, Special Publication No. 19. Oostende, Belgium. pp 296.

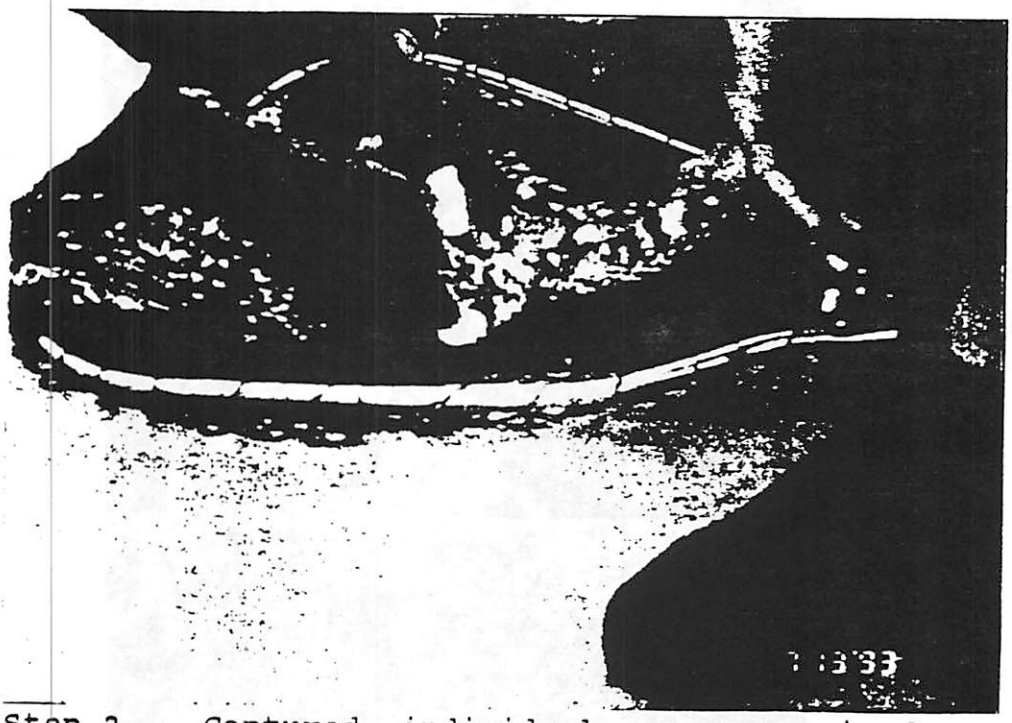
Tamaru, C.S., W. J. FitzGerald and V.S. Sato. 1993b. In: Christine Carlstrom-Trick (Ed.), Hatchery manual for the artificial propagation of striped mullet (*Mugil cephalus*). Guam Aquaculture Development and Training Center. 165 pp. (In press).

APPENDICES

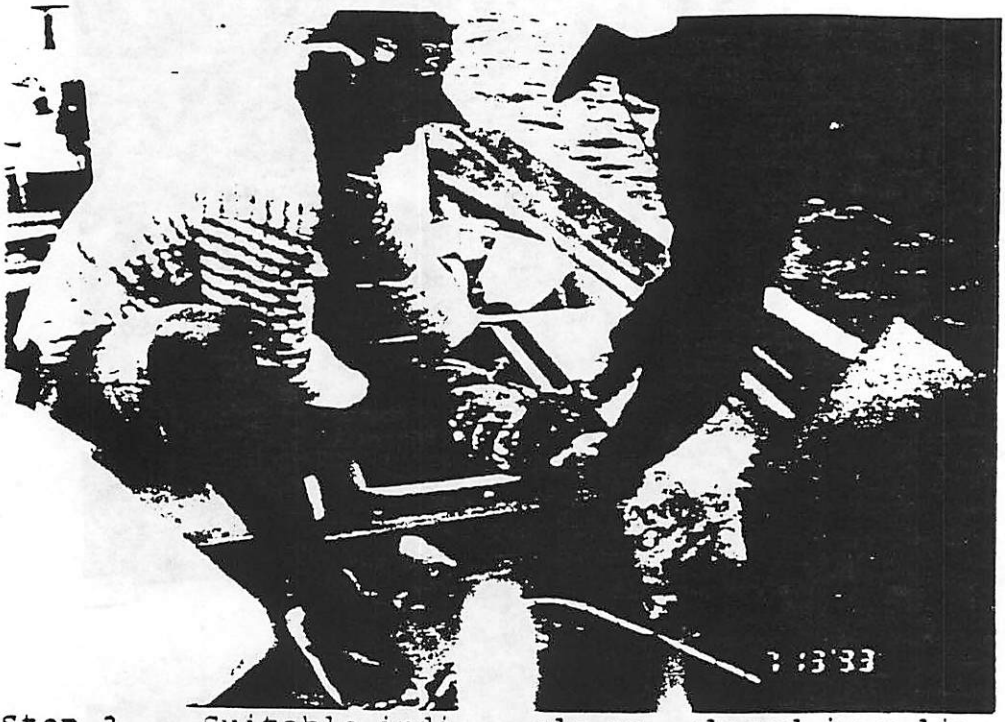
APPENDIX 1.
PICTORIAL ESSAY OF THE METHODS OF CAPTURE, TRANSPORT, INDUCTION OF SPAWNING, AND LARVAL REARING TANKS



Step 1. Adult groupers are obtained by hook and line.



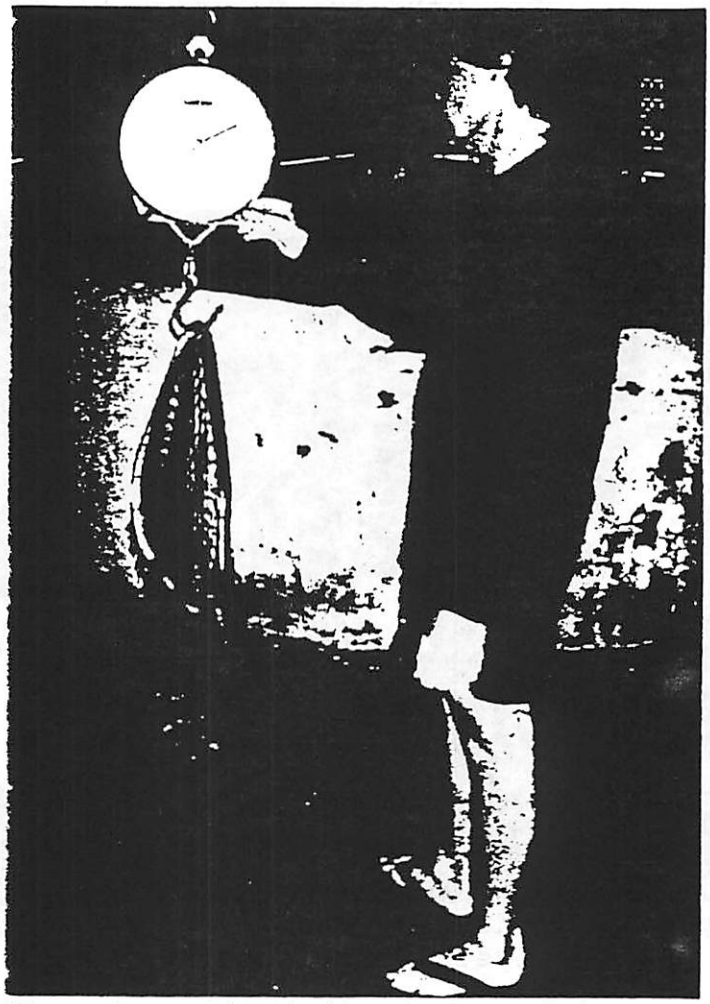
Step 2. Captured individuals are examined on board to assess their state of maturity.



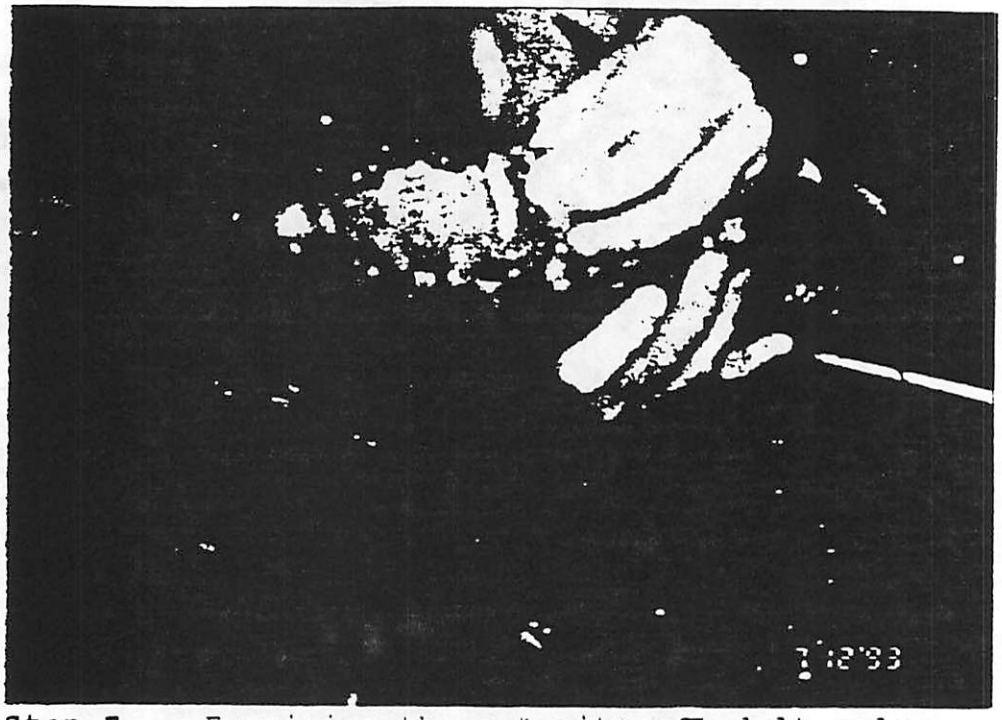
Step 3. Suitable indi als are placed in a live bait well for ..nsport.

Step 4. Unloading adult groupers.

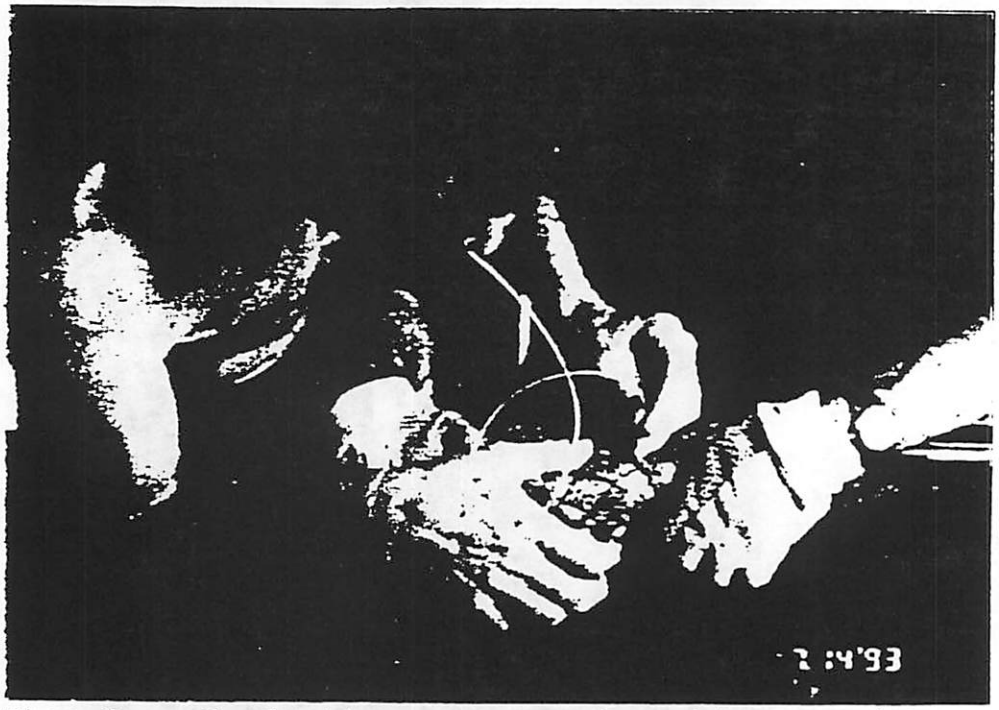
Step 5. Removal of air from the coelomic cavity of captured groupers.



Step 6. Obtaining body weight.

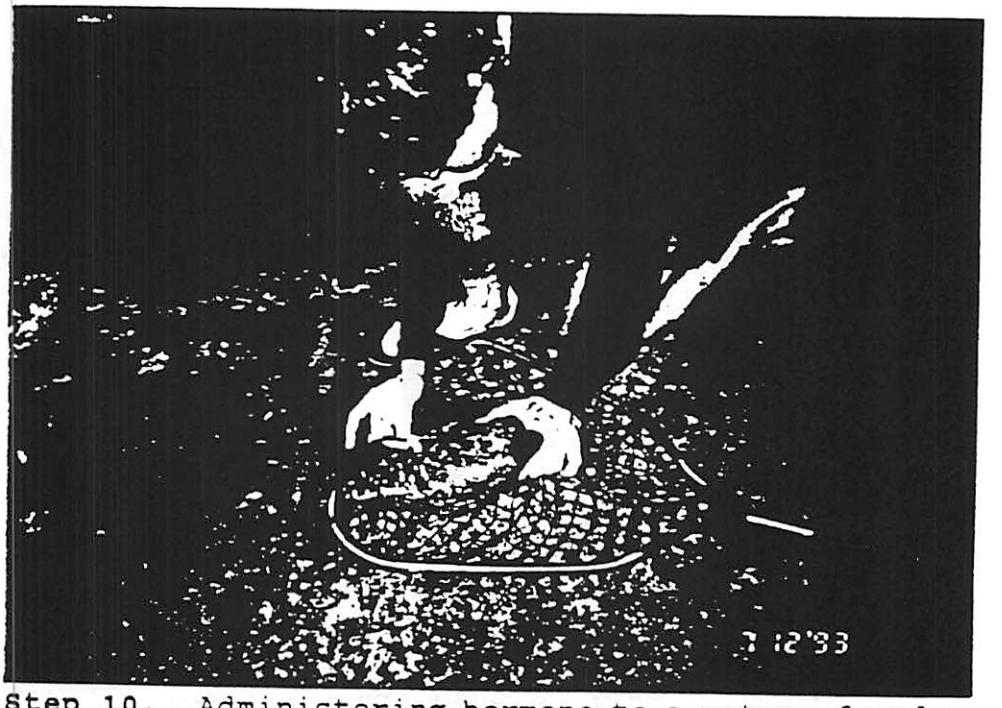


Step 7. Examining the maturity of adult males.

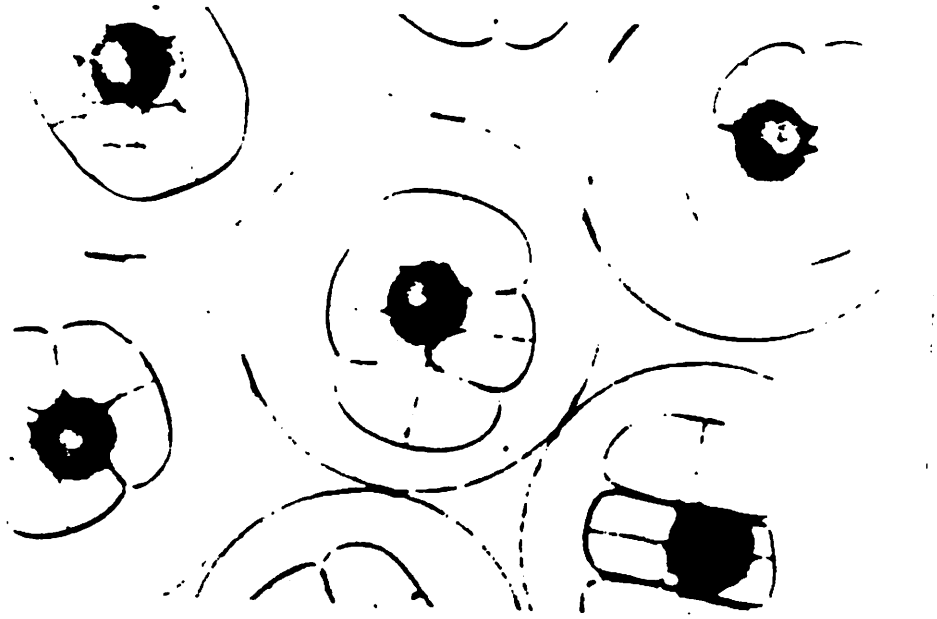


Step 8. Conducting an ovarian biopsy with a polyethylene cannula.

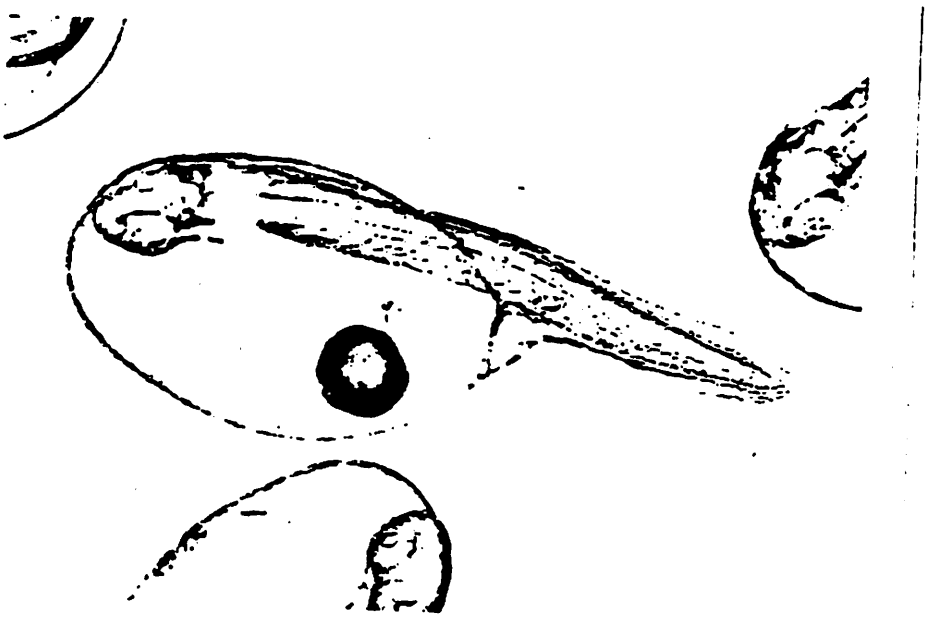
Step 9. Preparation of -hormone for the induction of spawning.



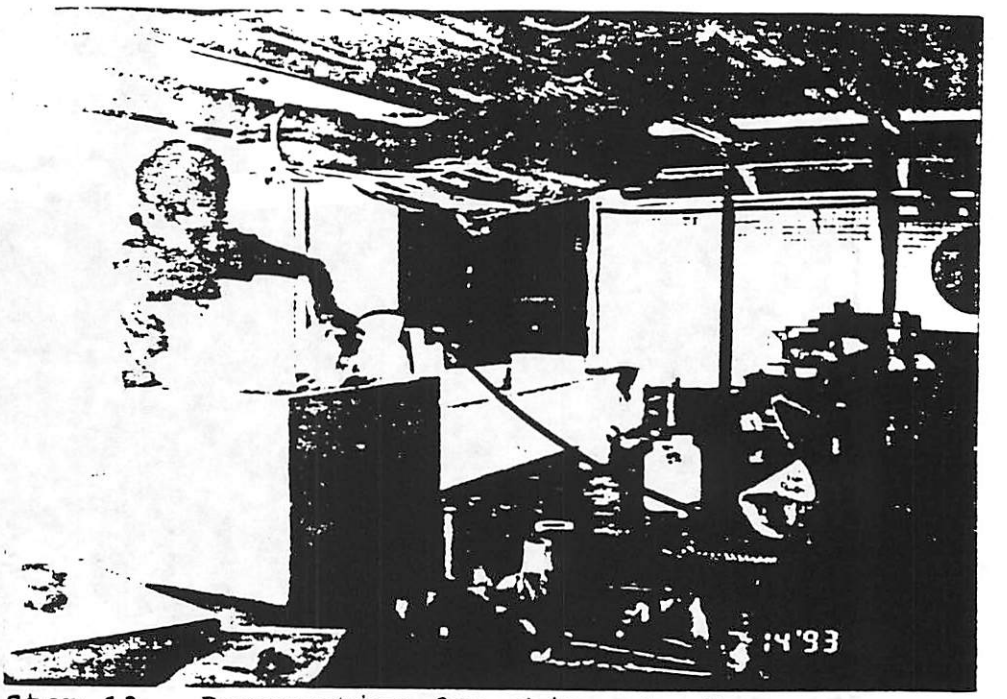
Step 10. Administering hormone to a mature female.



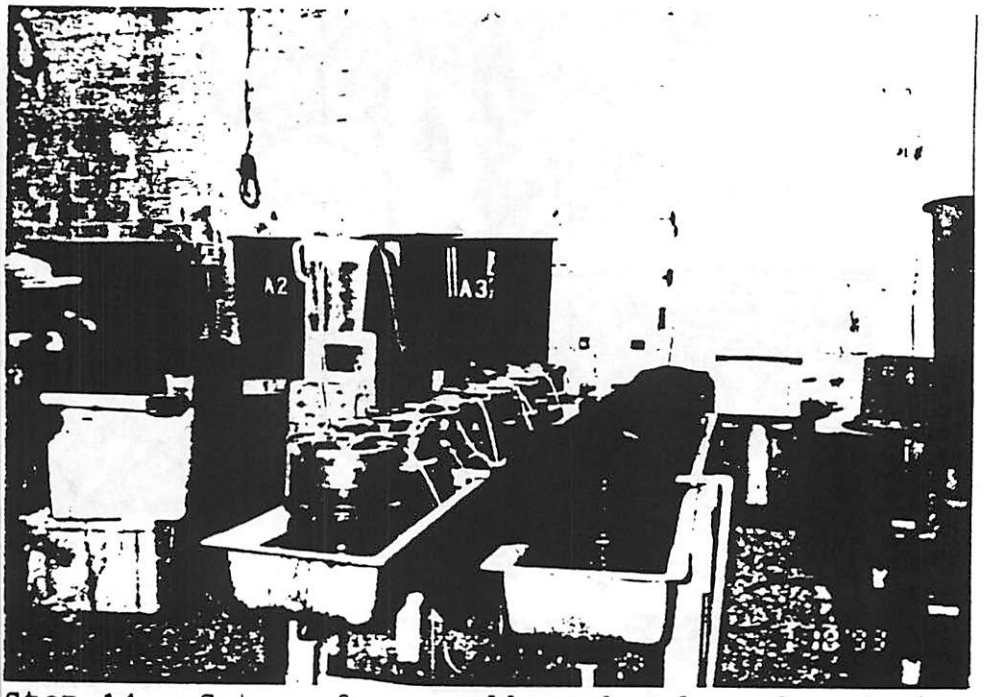
Step 11. Fertilized eggs from *E. microdon*.



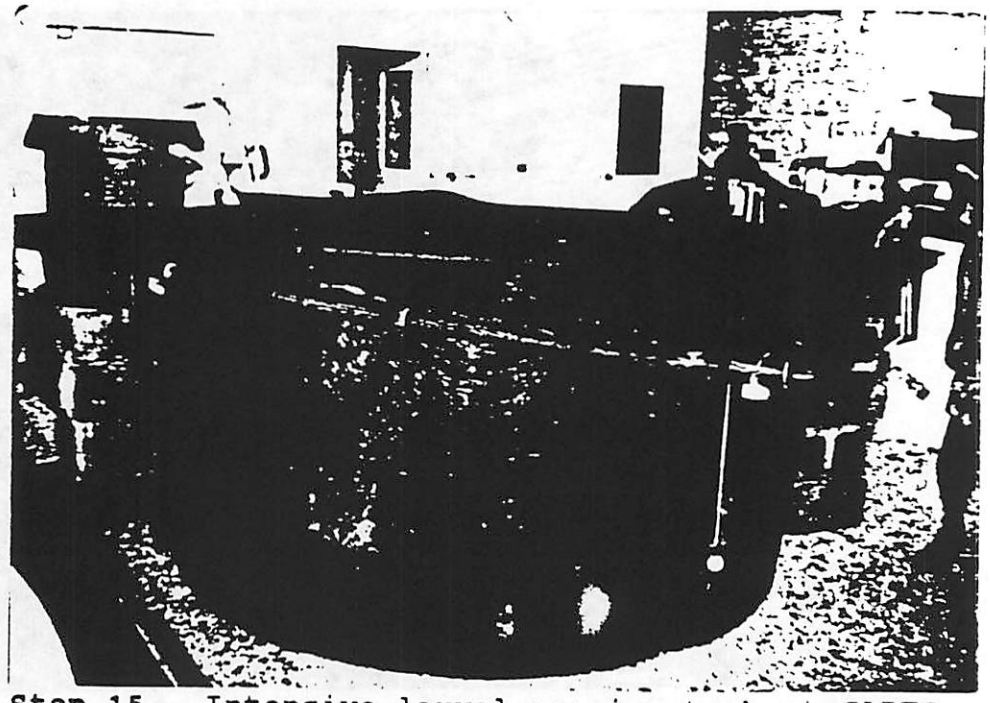
Step 12. Newly hatched grouper larvae.



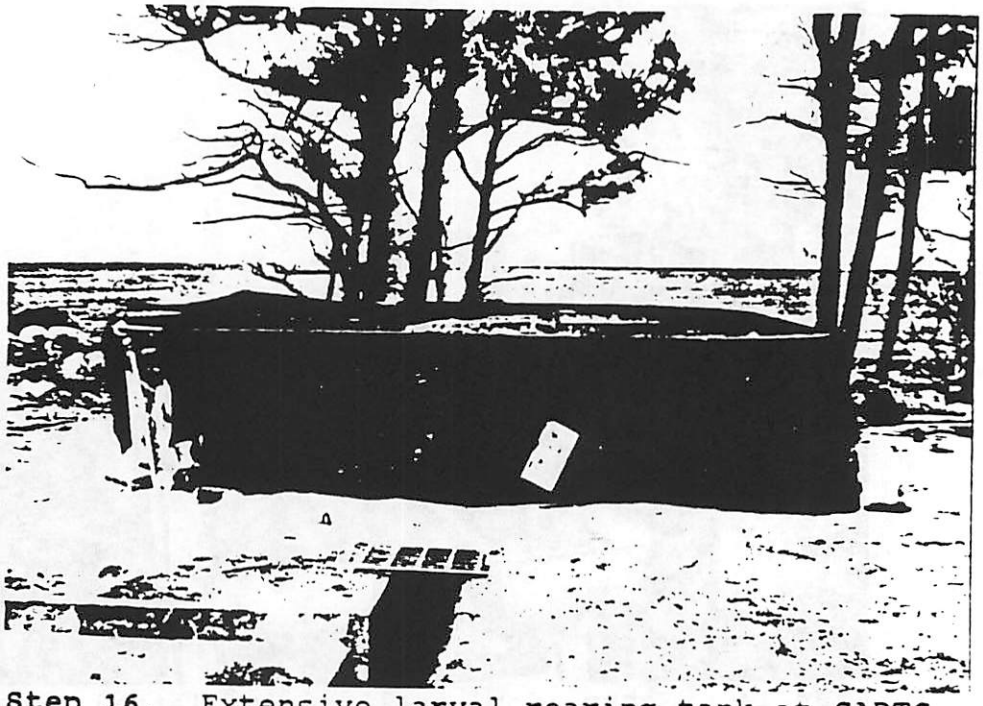
Step 13. Preparation for shipment of grouper eggs to GADTC.



Step 14. Setup for small-scale larval rearing experiment at GADTC.

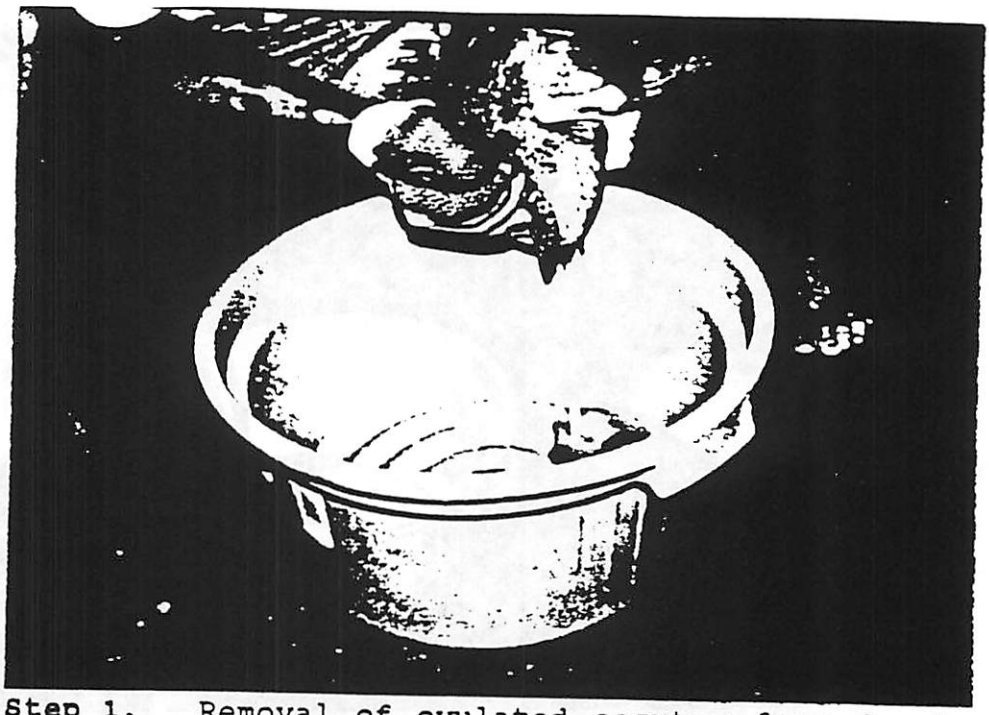


Step 15. Intensive larval rearing tank at GADTC.



Step 16. Extensive larval rearing tank at GADTC.

APPENDIX 2.
PROCEDURE FOR STRIP SPAWNING AND "DRY METHOD" OF FERTILIZING EGGS

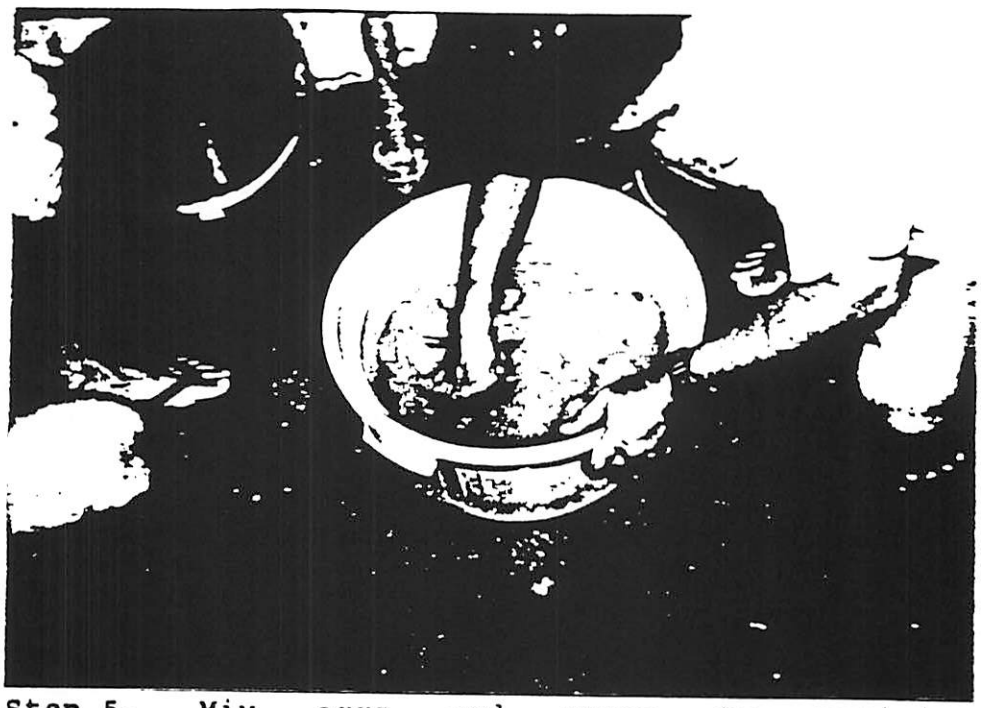


Step 1. Removal of ovulated oocytes from female by applying pressure to the abdomen.

Step 2. Distribute collected sperm with syringe. Note there is no seawater.

Step 3. Mix eggs and sperm with your hands.

Step 4. Activate sperm by adding seawater.



Step 5. Mix eggs and sperm to maximize fertilization.

Step 6. Skim the floating (i.e., fertilized) eggs with a beaker.

APPENDIX 3
SPAWNING BEHAVIOR OF *EPINEPHELUS MICRODON*

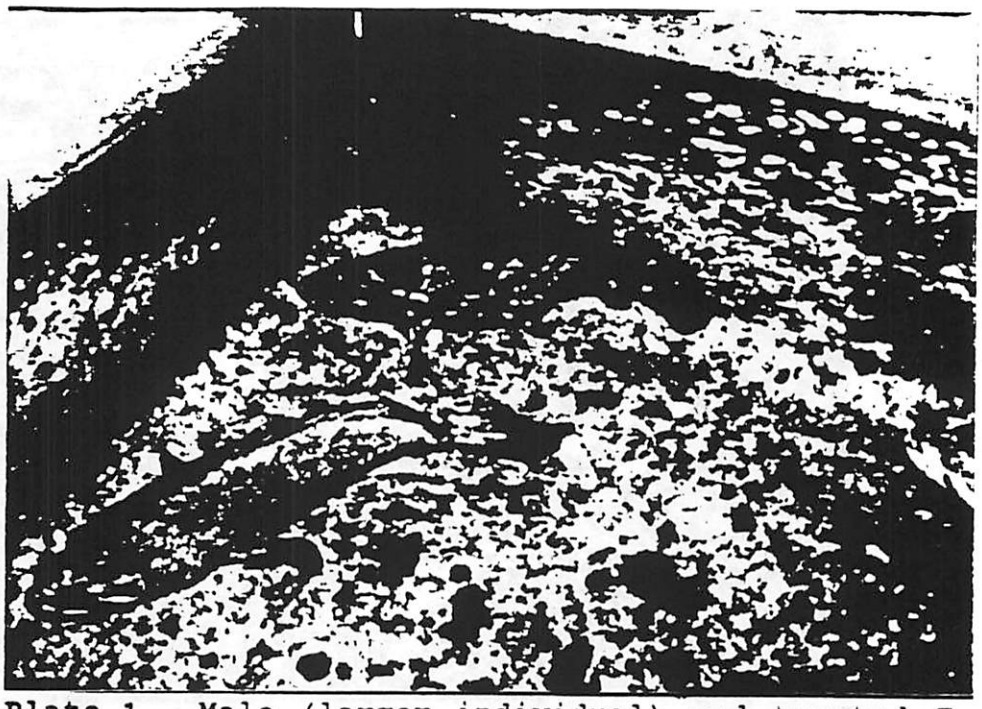


Plate 1. Male (larger individual) and treated *E. microdon* female after receiving the second injection. Note the distended abdomen of the female.

Plate 2. Swimming of female in the spawning tank as spawning approaches. Note the proximity of the males.

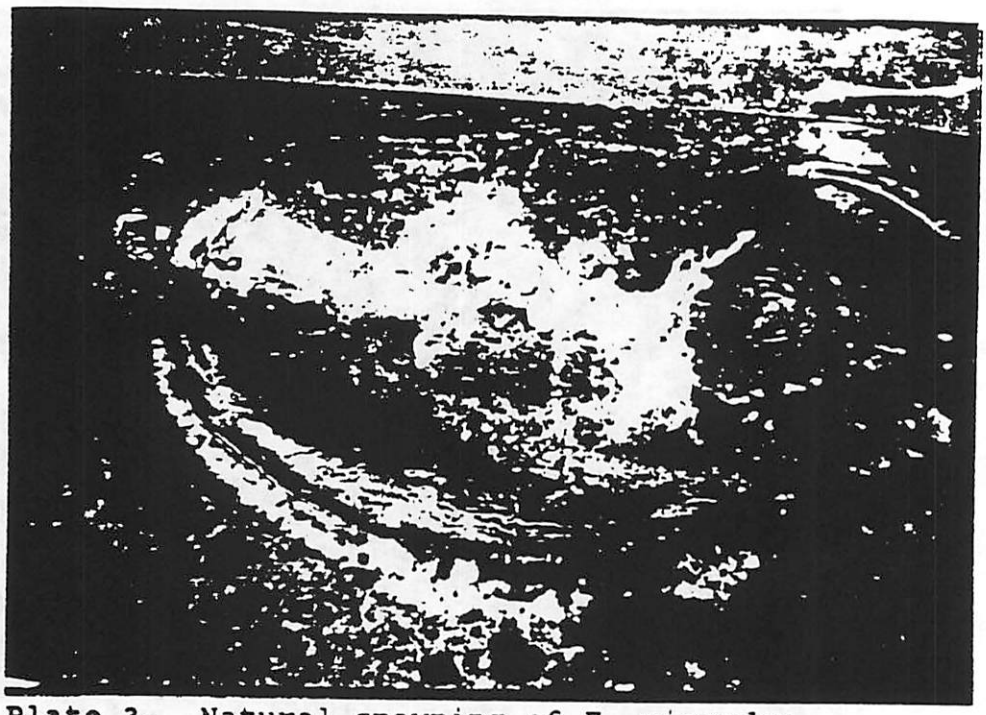
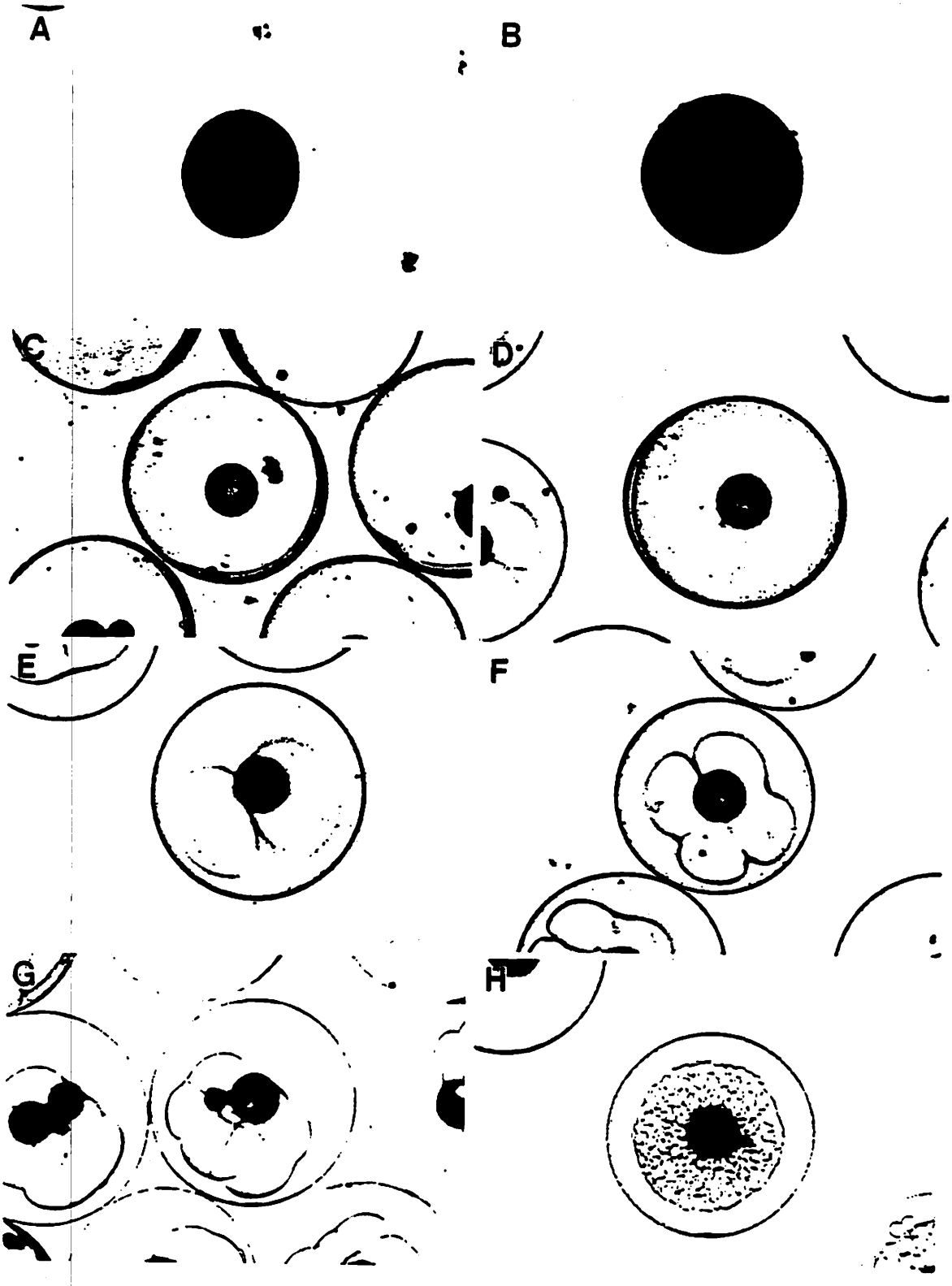


Plate 3. Natural spawning of *E. microdon*

Plate 4. Egg collector for spawned eggs of *E. microdon*

APPENDIX 4
TEMPORAL CHANGES OF OOCYTES DURING FINAL MATURATION, SPAWNING, AND EMBRYONIC DEVELOPMENT FOR *EPINEPHELUS FUSCOGUTTATUS*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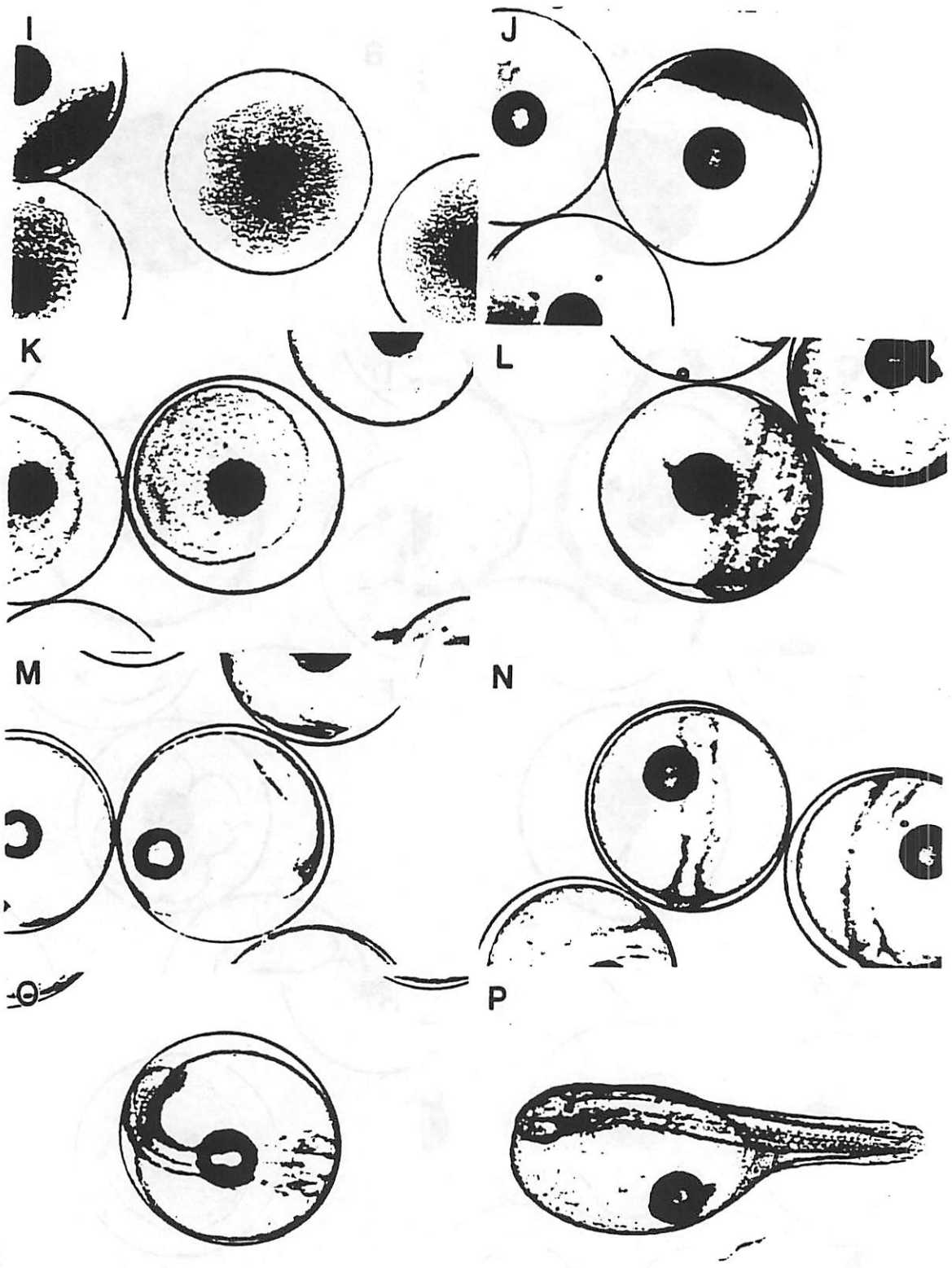
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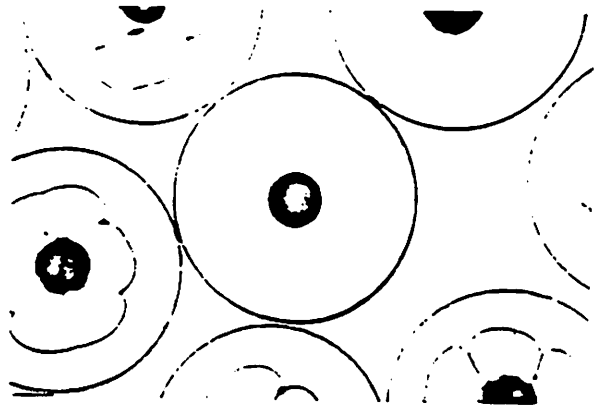


Plate Q. Example of a "bad" spawned grouper egg.

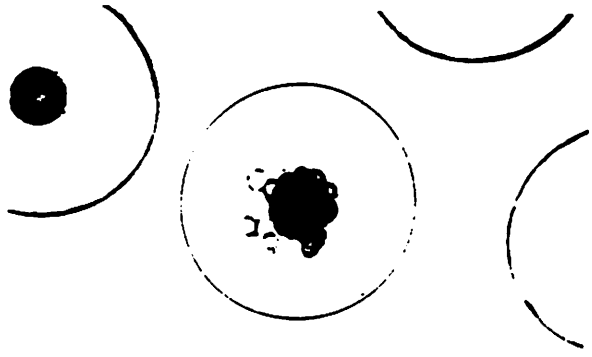


Plate R. Example of "bad" spawned grouper egg.

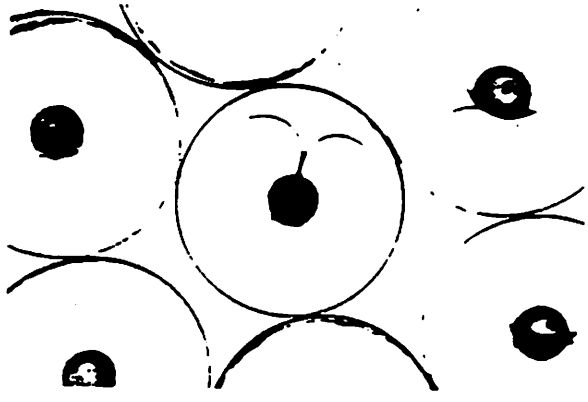


Plate S. Fertilized "good" eggs.

APPENDIX 5
MORPHOLOGICAL CHANGES OF *EPINEPHELUS FUSCOGUTTATUS* LARVAE

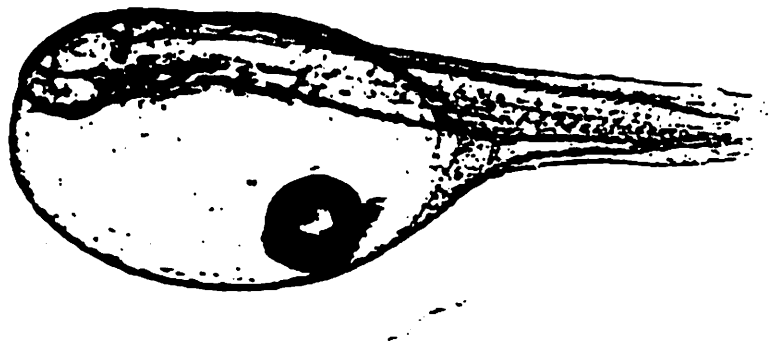


Plate 1. Newly hatched *E. fuscoguttatus* larvae.

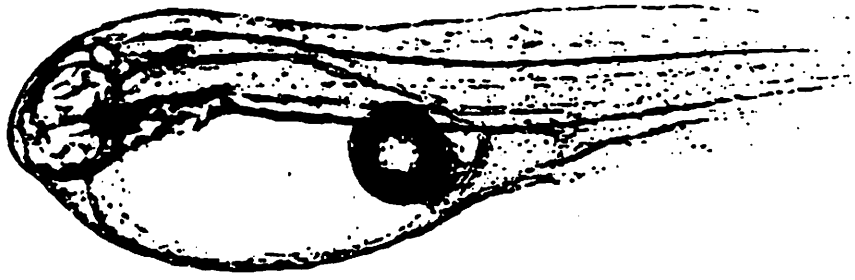


Plate 2. *E. fuscoguttatus* larvae seven hours posthatching.

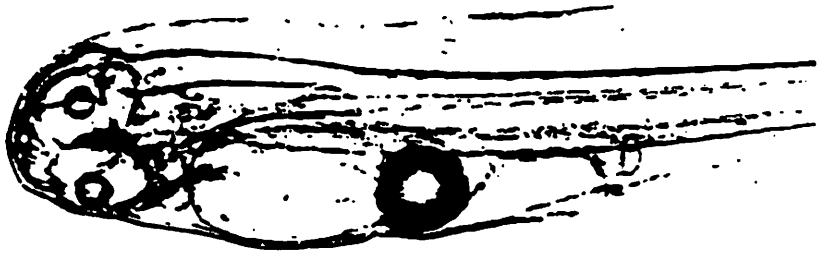


Plate 3. *E. fuscoguttatus* larvae, 24 hours posthatching.

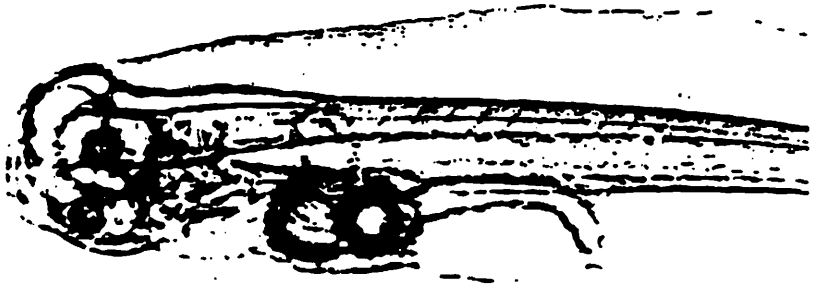


Plate 4. *E. fuscoguttatus* larvae, 48 hours posthatching.

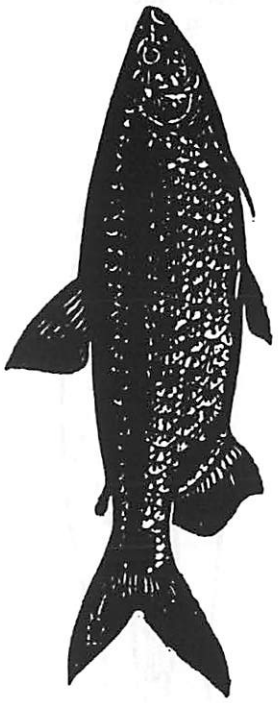
Plate 5. Four day posthatched *E. fuscoguttatus* larvae from extensive larval rearing tank.

Plate 6. Closeup of head region of 4 day old *E. fuscoguttatus* larvae. Mouth opening is 150 μm.

APPENDIX 6
OYSTER TROCHOPHORES AND A COMPARISON OF ROTIFERS TO TROCHOPHORES AND COPEPODITES

TrochoFeed-Back

"Besides saving us money, having the 18-liter container with frozen *Trochofeed* on hand all the time has made feeding schedules much more flexible. Now I can feed as little or as much as I want, whenever I want, and since the need for trochophores changes depending on which animals I'm trying to raise, that flexibility is important"



"This past spawning season we fed your *Trochofeed* to larval sablefish (black cod), and not only were the stomachs of the larval fish full of trochophores, it was also evident that the trochophores were in an advanced stage of degradation from digestion"

"We have been fortunate enough to be able to experiment with a wide variety of different food types and sizes. Your *Trochofeed* product has proven to be very promising as a first food offering for the fry in their early stages of development"

"A prey preference study showed (that) red drum selected trochophores over rotifers during the first two days of feeding"

"On analyzing your oyster trochophores with about 15% 20:5n3 (EPA) and 15% 22:6n3 (DHA) it was evident your product provided many of the nutritional requirements for initial feeding of larval fish"

For more information on *TrochoFeed*, contact

MTL Biotech Limited
P.O. Box 5760, Station B
Victoria, B.C. Canada V8R 6S8

Tel: (604) 598-6019
Fax: (604) 598-2047
Cellular: (604) 744-9850

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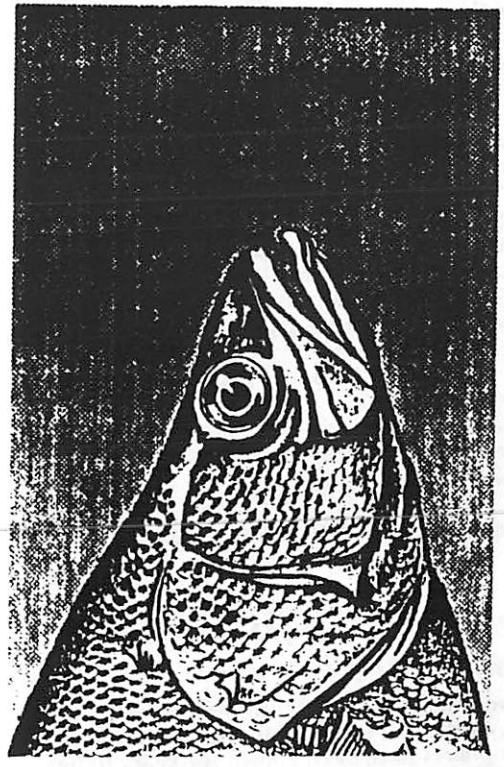
M T L
BIOTECH LTD

TrochoFeed



A new, natural first-feed for larval marine fish

TrochoFeed. . .

A new, natural first feed for larval marine fish

❑ What is TrochoFeed?

TrochoFeed is a suspension of living trochophore-stage Pacific oyster larvae in seawater, cryopreserved in liquid nitrogen until the moment they're needed to feed your fish. Trochophores are free-swimming and about 50µ in diameter - about a quarter the size of the average rotifer, and therefore an ideal first feed for larval marine fish. *Trochofeed* is the first and only "instant live feed" for marine larval rearing, and we have an impressive list of satisfied users.

Some of our customers. . .

- The Monterey Bay Aquarium
- The Vancouver Aquarium
- Pacific Biological Station, *Nanaimo, B.C., Canada*
- St. Andrews Biological Station
- The Huntsman Marine Science Centre
- Hawaii Institute of Marine Biology
- The National Aquarium in Baltimore
- The National Marine Fisheries Service
- Caribbean Marine Research Centre
- Mystic Marinelife Aquarium
- Point Defiance Zoo and Aquarium
- Texas Parks and Wildlife
- Marine Science Institute, *The University of Texas*
- Sea World of Ohio
- Mote Marine Laboratory
- Tulsa Zoo and Aquarium
- The John G. Shedd Aquarium

❑ Why do larval fish need TrochoFeed?

It's well known that live first feeds out-perform artificial diets, but they are labour-intensive to culture or collect. Oyster trochophores have been known for years to be an excellent first feed for larval marine fish; until now, however, the difficulty of obtaining them has made them an impractical diet

❑ Availability: The unique advantage of TrochoFeed

TrochoFeed is available every day of the year, thanks to **MTL Biotech Limited's** unique technology for cryopreserving the trochophores in bulk. We freeze trochophores during oyster spawning season, store them in liquid nitrogen and then ship them to you, frozen in bulk, whenever you want them. Frozen-thawed trochophores are alive and identical to fresh one: fish cannot tell the difference.

❑ High Food Value

The most important highly unsaturated fatty acids, 20:5n3 and 22:6n3, are each present at 15% of total fatty acids in *TrochoFeed*. Ash content is low because shell formation has not begun: we ship *TrochoFeed* to you at the peak of its nutritional value.

❑ What does TrochoFeed look like? How is it used?

Trochofeed is packaged in plastic straws that must be stored in liquid nitrogen until you are ready to feed (we can recommend a suitable liquid nitrogen container). Straws contain between 5 million and 100 million trochophores each, so you can choose which to thaw depending on your daily needs. Thawing is a simple procedure, requiring only a few litres of sea water and a 20µ screen. You can feed the trochophores over a wide temperature range: Arctic cod eat them as well as red drum do.

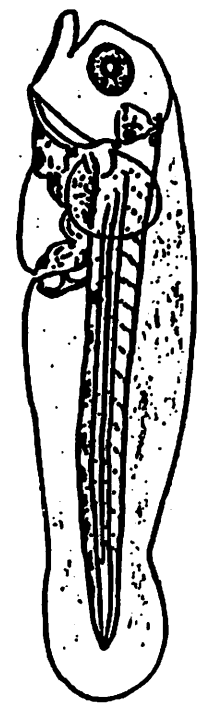
Live Larval Feeds (to scale)

1 2 3

1 *Artemia* nauplius, 2 Rotifer, 3 Trochophores

❑ Quality assurance and environmental safety

Trochophores are produced from certified, disease-free selected broodstock oysters and are cultured under controlled conditions in our facility. Users in areas where Pacific oyster is an "exotic" species need not fear that uneaten trochophores in effluent water will become established locally: our cryopreservation process ensures that the trochophores cannot complete normal embryonic development.



❑ Shipping TrochoFeed

Your order of frozen *TrochoFeed* is shipped to you, by air cargo, in a pre-chilled "dry shipper". Simply transfer the frozen *TrochoFeed* to your own storage container and send the shipper back to us. If you do not have a storage container, **MTL Biotech Limited** can rent you one.

❑ Ordering

Contact **MTL Biotech Limited's** sales department at:

MTL BIOTECH LTD

P.O. Box 5760, Station B

Victoria, B.C., Canada V8R 6S8

Tel: (604) 598-6049

Fax: (604) 598-2047

Cellular: (604) 744 9850

Guam Aqua 13 July 93

M T L
BIOTECH LTD

Invoice

Date: 13 July 1993

Sold to:

Dept. of Commerce
Guam Aquaculture Development Center
GITC Building, #601
Tamuning, GUAM
Attn: Mike Bauerlein/ Mark Norman
Tel: 671-646-5841; 734-3011

Purchase Order:
Via: Canadian Airlines

Item	Batch	Quantity	Unit price	Shipping	Cost
Trochofeed	14 Aug (green)	6 X 5 million	5.00	collect	150.00
Trochofeed	102 (blue)	1.5 X 13 million	5.00	collect	100.00
Trochofeed	101 (blue)	0.5 X 15 million	5.00	collect	N/C
Trochofeed	109 (blue)	1 X 35 million	5.00	collect	175.00
Trochofeed	103 (blue)	1 X 15 million	5.00	collect	75.00

DISCOUNTS AVAILABLE ON LARGER ORDERS

Please pay: 500.00

P.O. Box 5760, Station B, Victoria, B.C., Canada, V8R 6S8
Tel: (604) 589-6019 Fax: (604) 598-2047

HOW TO THAW AND RECOVER FROZEN TROCHOFEED

Important safety precaution

Always wear protective eyewear when handling cryopreserved Trochofeed. Cryogenic sample holders, including vials, tubes and straws, can shatter. Cryogenic storage vessels, including dewars and refrigerators, are supplied with manufacturer's safety guidelines, which should always be followed.

1. Selecting material

Select a straw of cryopreserved trochophores and place it **immediately** into the plastic thawing bag provided. *The bag is for convenience in thawing, and for your protection in the unlikely event of straw breakage.* Holding the *outside* of the bag, **immediately** snap the straw into 2-3 inch segments. If you're not using the whole straw, replace the unused portion in the liquid nitrogen storage container **immediately**. *DO NOT ALLOW UNUSED PORTION TO WARM UP, AS DAMAGE TO THE REMAINING MATERIAL WILL OCCUR.*

2. Thawing

Add warm seawater (25-28C) to the bag *without delay,* using about 300 ml per straw. Shake gently, squeezing the Trochofeed out with your fingers to achieve a rapid thaw. The straw pieces must be submerged to get the right thaw rate. Remove empty straw pieces.

3. Recovery period

Filter the thawed trochophores over a 20µ Nitex screen, wash with a few squirts of 25-28C seawater. then rinse them into a container of 25-28C seawater for an appro ately 1-2 hour recovery period. Density during the recovery period should be *roughly* 400 ml ater per million trochophores (2,500 trochophores/ml). After one hour, harvest the trochophores on the 20µ screen as before, then rinse them directly into the rearing tanks for feeding.

5. Counting trochophores

Your packing list or invoice tells you *roughly* how many trochophores are contained in each straw. If you need more accurate counts, dilute the washed trochophores to an easily counted density (10,000 trochophores/ml works well) and count using a Sedgwick-Rafter cell at 40X magnification.

6. Thaw too many?

Unused trochophores will keep overnight in a refrigerator (4-5C).

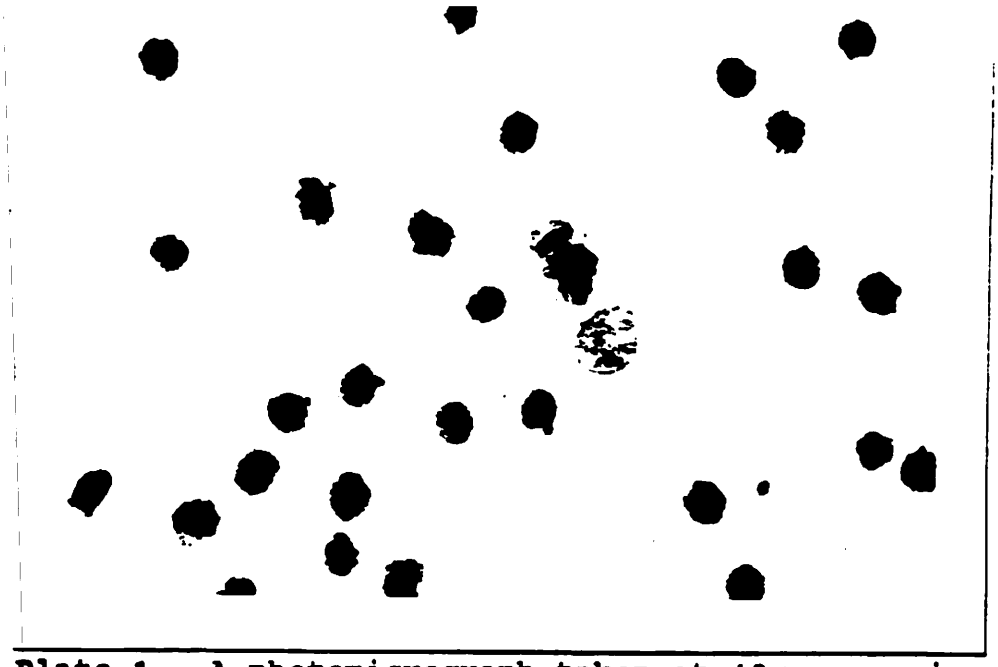


Plate 1. A photomicrograph taken at 40x comparing a rotifer and oyster trochophores.

Plate 2. Newly hatched *Artemia* nauplii.

Plate 3. Adult herpactocoid copepods from shrimp broodstock tanks. Note female carrying eggs.

Plate 4. A photomicrogaph of a rotifer and copepodite. 40x.

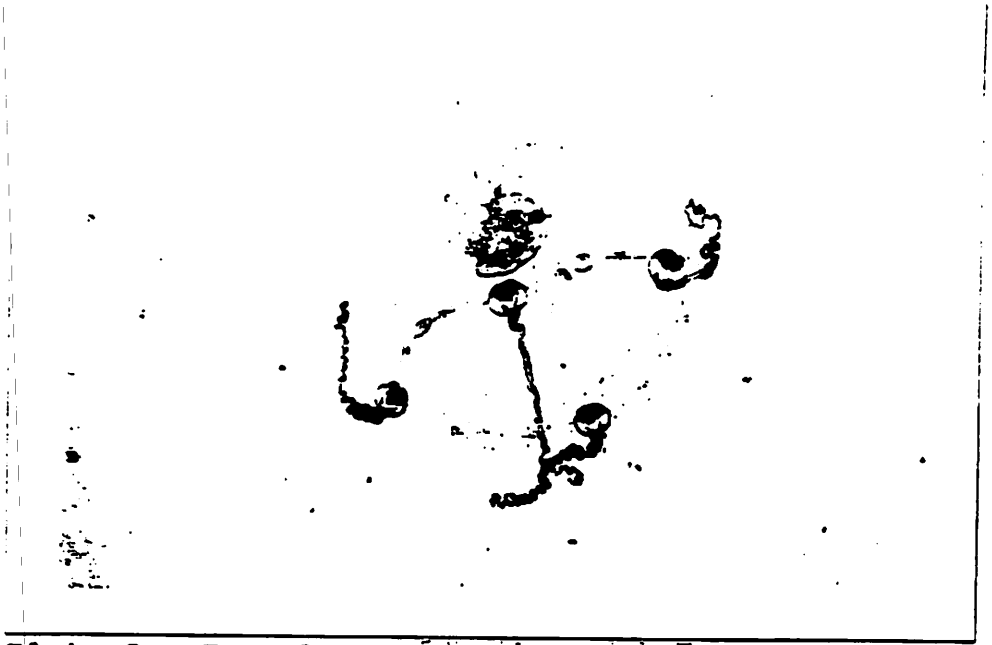
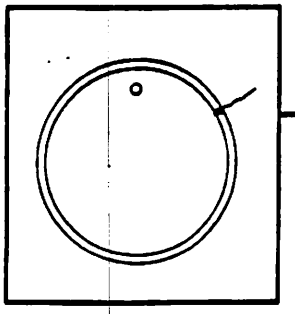


Plate 5. Example of medusa present in the extensive larval rearing tank.

APPENDIX 7
SPAWNING PROTOCOL FOR THE SEA URCHIN, *Tripneustes gratilla*



Exercise 2

Sea Urchin Fertilization

Introduction

Sea urchin gametes have been used extensively in studies of fertilization because they are easily obtainable in large quantities and develop *in vitro*. Much of what we know about the physiology and biochemistry of early development has come from studying sea urchin embryos. The early literature was well summarized by Harvey (1956); more recent reviews by Giudice (1973) and Stearns (1974) provide insight into an array of research efforts involving these animals. Modern research with these animals involves primarily molecular biology.

This exercise will provide you with an opportunity to learn how to acquire, examine, and manipulate sea urchin gametes to produce embryos of various stages for subsequent study.

Procuring and Storing Gametes

Objective

☐ Know how to induce spawning, and how to properly wash and store gametes.

Sexes can be distinguished only by examining the reproductive products; milky semen and yellow (or orange), granular eggs. Gametes may be obtained by several methods:

- an animal may be cut open and the gonads removed and minced in sea water;
- spawning may occur spontaneously in the laboratory (often due to stress);
- spawning may be induced by electric shock, or injection of 0.55 *M* KCl solution into the coelomic cavity. Both induction methods induce smooth muscle contraction, causing gametes to ooze from the five gonopores on the aboral surface.

In this course, spawning will be induced by injection of 0.55 *M* KCl solution into the coelomic cavity. With a hypodermic needle mounted on a syringe, puncture the soft perioral membrane, pointing slightly away from the oral–aboral axis and into the coelomic cavity. Use 1–5 cc of solution, depending on the animal's size and initial response. To avoid contamination of gametes, use a separate syringe and needle for each animal, or rinse the instruments well in tap water after each use.

Examine the gametes shed at the aboral surface and determine the sex of the animal; semen is creamy white, whereas eggs are finely granular and yellow or orange. Females should be inverted over a beaker of filtered sea water (FSW) such that the gonopores are submerged and the eggs stream out without exposure to air. Collect semen "dry" (without dilution) by inverting the spawning male over a dry petri dish. Remove any excess fluid that drips from the animal into the semen by pipet. Cover the dish to prevent desiccation until the sperm are to be used.

Eggs should be washed promptly because coelomic fluid hinders fertilization. When the female no longer sheds gametes, remove her and gently swirl the beaker to suspend its contents. Permit the heavier "crud" (bits of spine, tube feet, etc.) to settle for about 30 seconds and then decant the suspended eggs into another beaker. The eggs

will settle to the bottom; the supernatant, called egg water, should be decanted (save for later use) and replaced with clean sea water. This washing process should be repeated at least twice.

> Hint: Small quantities of eggs may be washed by moving in a circular pattern on a table top a small petri dish containing an egg suspension. Eggs collect in the middle of the dish where they may be removed by pipet to a dish of clean sea water. Alternatively, small quantities of eggs may be suspended in sea water in centrifuge tubes and quickly spun down by hand centrifugation. The wash water is then easily decanted and replaced.

Undiluted ("dry") sperm may be kept in a closed container in the refrigerator (about 5°C) and used successfully up to 48 hours after collection. Washed eggs (if not extremely crowded) may be stored cold up to about 6 hours without appreciable deterioration. Thereafter, the percent of eggs fertilizable declines markedly.

Examining Gametes

Objective

- ☐ Learn to determine the fertility of a sample of gametes.

Sea urchin eggs are usually about 70-100 µm in diameter and are surrounded by a 50 µm-thick jelly coat. Immature oocytes have a conspicuous large nucleus (germinal vesicle). Most of the eggs (ova) will have completed both maturation (meiotic) divisions and may have polar bodies adhering to the animal pole (difficult to detect). The nucleus of the mature ovum is small, but conspicuous.

Spermatozoa consist of a cone-shaped head about 2 µm long and 0.5 µm in diameter at the base, where several mitochondria form the midpiece. A 50 µm- long tail projects from the midpiece. Spermatozoa are relatively inactive in the undiluted ("dry") state, but become active immediately upon dilution with sea water and are soon exhausted. Because of this activating dilution effect one should always use a fresh (less than 10 minutes old) dilution of semen when fertilizing eggs. Once diluted in sea water, spermatozoa are fertile only about 20–30 minutes. (Low pH retards activation; high pH accelerates it.)

Obtain some gametes and examine them closely before mixing sperm and eggs.

- Are the sperm active?
- Are the eggs uniform in size, round, and free of contaminants?
- What proportion of the eggs are mature? Do they touch each other? Why?
- Are any of the eggs fertilized prematurely?

These questions should be considered each time you procure gametes for use.

Fertilization

When sperm and eggs are brought together in the right conditions fertilization occurs. The most immediately recognizable changes occur at the egg surface; darkfield microscopy reveals there an inconspicuous alteration of the color of the diffraction rings. Within seconds of sperm entry the egg produces a fertilization membrane which rises above the egg surface; it is more prominent in some species (e.g., *Pseudoboletia*) than in others (e.g., *Tripneustes*).

A small protrusion (the fertilization cone) may be seen where the sperm head enters the egg cytoplasm, but only from a (relatively rare) side view. The male pronucleus moves to the female pronucleus and the nuclear membrane of each disappears. The appearance of the cytoplasm slowly changes as the mitotic cleavage spindle forms and karyokinesis (nuclear division) progresses. Ultimately, cytokinesis (cytoplasmic division) commences and very quickly the single-celled zygote divides into two blastomeres.

Achieving Optimum Fertilization

Objective

☐ Learn to recognize fertilization changes in gametes and how to maximize yields of viable embryos.

Determine the dilution of semen which gives optimum fertilization by adding equal numbers of eggs to a series of dishes containing serially diluted sperm. To do this, add equal volumes (e.g., 2 ml) of filtered sea water to a row of labeled plastic petri dishes. Make a stock suspension of sperm by adding one drop of "dry" semen to 50 ml of FSW in a beaker. With a pipet, transfer a volume (2 ml) of this stock suspension into an equal volume of FSW in the first dish to produce a ½ dilution. Then transfer half of this dilution into the next dish of FSW to give a ¼ dilution, and so on. To each dish add an equal volume* of eggs (e.g., 1 drop) and examine for evidence of fertilization (elevation of fertilization membranes). There should be too many sperm in the first dilution (leading to polyspermy and abnormal development) and too few in the last (decreased fertilization rate). If not, extend the dilution series.

The number of sperm in the stock suspension can be estimated by measuring its optical density with the colorimeter and comparing the result with a standard curve. With this information you can estimate how many sperm are required to fertilize an egg.

Using a format similar to Table 2.1, record the percentage of mature eggs fertilized in each sperm dilution, based on elevation of fertilization membranes. Count at least 100 eggs in several microscopic fields of view. Cover the dishes to prevent desiccation and count the percentage of cleaved eggs later. How do these counts compare with those based upon fertilization membrane elevation? (If you really know how to recognize fertilization membranes, the two percentages for each dilution will be the same.)

Note: What you seek from this exercise is the sperm concentration which yields optimum fer-

Table 2.1. Optimum sperm dilution for fertilization in *Tripneustes gratilla*.

Sperm Dilution	Drops "Dry" Semen per ml FSW	% Mature Eggs Fertilized	% Mature Eggs Cleaved
1	1/50	_____	_____
1/2	1/100	_____	_____
1/4	1/200	_____	_____
1/8	1/400	_____	_____
1/16	1/800	_____	_____
1/32	1/1600	_____	_____
1/64	1/3200	_____	_____
1/128	1/6400	_____	_____

* The exact number of eggs is unimportant, but precision (repeatability) is important. As a start, hold a pasteur pipet filled with egg suspension vertically until the eggs collect into the tapered portion, then deliver one drop of this compact suspension into the water in the dish. (How would you determine the mean number of eggs delivered by this method? What effect does repeated washing of the eggs have upon this technique?)

tilization; i.e., the fewest sperm required to fertilize all of the mature eggs.

Timing Normal Development

Objective

- ☐ Produce a timetable for normal development at standard laboratory conditions.

In order to use embryos for experimentation later in this course, you need to predict the time of appearance of a particular stage of development following fertilization. This will require that you produce a developmental timetable such as that shown in Table 2.2.

Rate of development varies with such factors as temperature, pH, salinity, and even individual variability. For this course, standard conditions will be room temperature (20°–22°C) and filtered sea water (pH 7.8–8.3; 35 °/oo salinity).

Time to achieve a particular stage of development is recorded when 50% of the sample is at that stage. This will require your attention following fertilization to note when the first individuals enter into a particular stage, and constant monitoring (and counting) until 50% have done so.

Observing Fertilization

While you are waiting for cleavage to begin, put some washed, unfertilized eggs on a slide and examine them in detail. To prevent crushing the eggs with the coverslip, prop it up with pieces of broken coverslip, grains of sand, a hair, or some clay or wax on the corners ("footed coverslip"). Examine spermatozoa in the same manner. Add some diluted sperm to one side of the egg preparation and watch the sperm swim to the eggs and activate them. Make sure you can recognize fertilization membrane elevation.

Table 2.2. Timetable for normal early development of *Tripneustes gratilla*.

Location: _____ **Temperature** _____

Stage	Date/Time	Time After Fertilization to 50% at Stage
Gametes mixed		
Fertilization membrane		
Pronuclei fusing		
2–cell stage		
4– cell stage		
8–cell stage		
16–cell stage		
Blastula		
Hatching		
Gastrula		
Prism		
Pluteus		

Specific Interacting Substances of Eggs and Sperm

Objective

☐ Demonstrate that eggs and sperm have chemical properties which contribute to specific interaction during fertilization.

F. R. Lillie (1912) demonstrated that ripe eggs of sea urchins release a substance, fertilizin, which activates and agglutinates spermatozoa of the same species. Fertilizin was believed to interact with a sperm substance, antifertilizin, in a manner important to the natural process of fertilization. The following exercises demonstrate some of the properties of these substances and (hopefully) will stimulate curiosity about the process of gamete activation.

A. Preparation of Egg Water Fertilizin. (Vasseur, 1948)

1. Wash unfertilized eggs one time in a small volume of FSW.

2. Extract the egg jelly coat by treating the eggs for 15–30 mins in an equal volume of acid SW (2 ml *N* HCl + 100 ml FSW, pH 5.5–5.8).

3. Sediment the eggs with the hand centrifuge and decant the jelly coat extract into another beaker. Save the eggs in FSW.

4. Adjust the extract to pH 8.0 with 1*N* NaOH.

B. Preparation of Sperm Water Antifertilizin. (choose one method)

1. Dilute "dry" sperm with an equal volume of FSW. Heat 10 mins at 60°C, then cool and centrifuge or millipore filter to remove sperm. (Frank, 1939).

2. Dilute "dry" sperm in an equal volume of acid SW and freeze. Thaw and centrifuge or millipore filter to remove sperm. Adjust pH to 8.0 with 1*N* NaOH (Tyler, 1939).

C. Agglutination and Activation Properties of Fertilizin. (Lillie, 1912)

1. Prepare a slide with a footed coverslip. Add a drop of diluted sperm to one side, and a drop of egg water to the other side of the coverslip. Observe what happens as the two materials mix and interact.

2. How might this property function in normal fertilization?

D. Testing Agglutinated Sperm Capacity for Fertilization. (Lillie, 1913)

1. Mix 2 drops of diluted sperm (1 drop "dry" sperm in 50 mls FSW) into 10 drops of egg water (egg jelly extract, from A) and allow to stand for 10 mins.

2. Set up and fertilize two dishes of normal eggs.

   a. Dish #1 fertilized with 1 drop of the above agglutinated sperm.

   b. Dish #2 fertilized with 1 drop of normal diluted sperm (1 drop "dry" in 50 ml FSW).

3. Observe percent fertilization in each dish and compare.

4. What role in normal fertilization might be played by this property?

E. Agglutination of Egg Jelly by Antifertilizin. (Frank, 1939)

1. Place 10 drops of sperm water antifertilizin (from B, above) in a small dish.

2. Add 1 drop of normal eggs (with jelly coats). Let stand for 10 mins.

3. Place a sample of the eggs on a footed coverslip mount and examine under oblique lighting for a precipitation ring at the outer edge of the jelly. Add a small drop of Toluidine Blue solution to accent the ring.

4. How do you account for this result?

F. Agglutination of Naked Eggs with Antifertilizin.
(Frank, 1939)

1. Place 10 drops of sperm water antifertilizin (from B, above) in a small dish.

2. Add 1 drop of naked eggs (jelly coat removed, from A, above). Let stand for 10 mins.

3. Place a sample of the eggs on a footed coverslip mount and examine.

4. How might this property function in normal fertilization?

G. Testing the Fertilizability of Naked Eggs.
(Lillie, 1915)

1. Prepare two dishes of eggs:

   a. Dish #1: normal eggs (washed once).

   b. Dish #2: naked eggs (jelly coats removed).

2. Fertilize each dish with normal diluted sperm and compare the results.

3. What might you conclude about the role of the jelly coat in the normal course of events in fertilization?

References

Frank, J. A. 1939. Some properties of sperm extracts and their relationship to the fertilization reaction in *Arbacia punctulata*. Biol. Bull. (Woods Hole) 76: 190–216.

Giudice, G. 1973. *Developmental Biology of the Sea Urchin Embryo*. Academic Press, N.Y.

Harvey, E. B. 1956. *The American Arbacia and Other Sea Urchins*. Princeton Univ. Press.

Lillie, F. R. 1912. The production of sperm isoagglutinins by ova. Science 36:527–530.

Lillie, F. R. 1913. Studies of fertilization. V. The behavior of the spermatozoa of *Nereis* and *Arbacia* with special reference to egg extractives. J. Exptl. Zool. 14: 515–574.

Lillie, F. R. 1915. Sperm agglutination and fertilization. Biol. Bull. (Woods Hole) 28: 18–33.

Tyler, A. 1939. Extraction of an egg membrane lysin from sperm of the giant keyhole limpet (*Megathura crenulata*). Proc. Natl. Acad. Sci. (U.S.) 25: 317–323.

Vasseur, E. 1948. Chemical studies on the jelly coat of the sea urchin egg . Acta Chem. Scand. 2: 900–913.

APPENDIX 8
VARIOUS GROUPER SPECIES ENCOUNTERED DURING THE CONSULTANCY

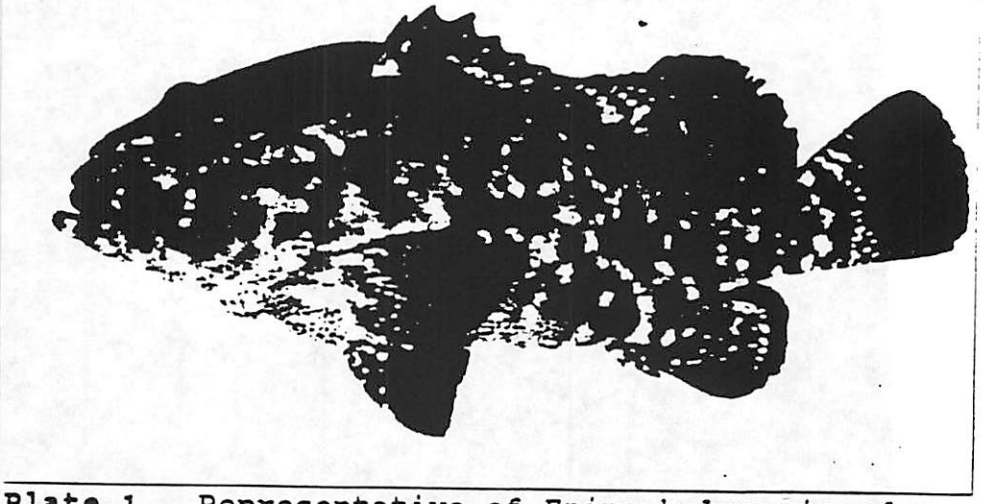


Plate 1. Representative of *Epinephelus microdon*.

Plate 2. Representative of *Epinephelus fuscoguttatus*.

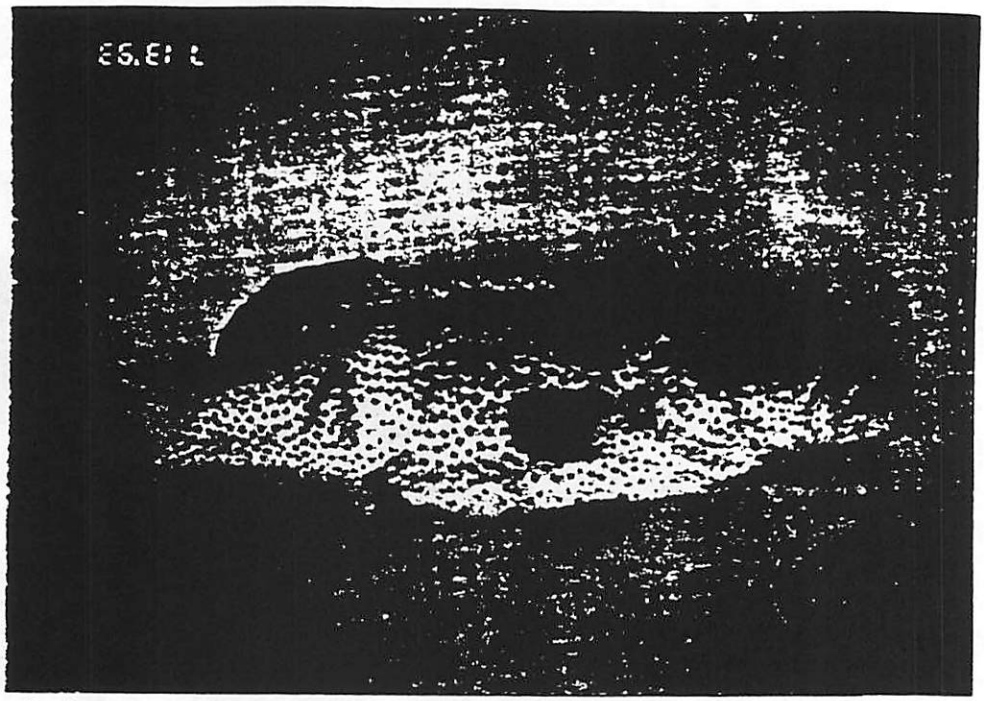


Plate 3. Representative of *Plectropomus aerolatus*.

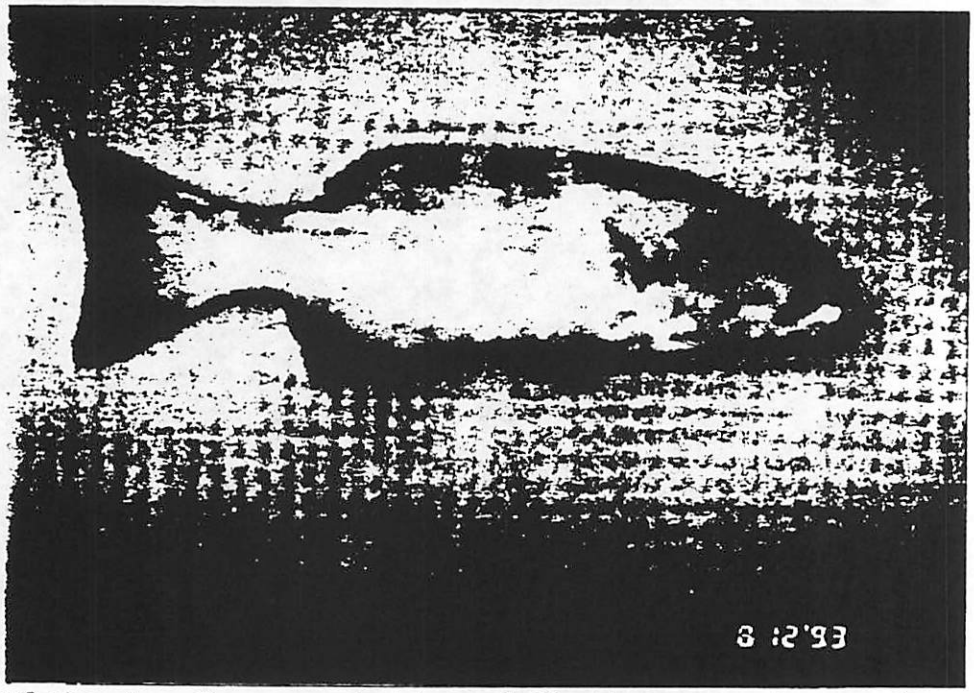


Plate 4. Representative of *Plectropomus leopardus*.

Plate 5. Example of *Cephalopholis miniata*.

Plate 6. Example of *Aethaloperca rogaa*.

APPENDIX 9
GONADAL TISSUE FROM *CEPHALOPHOLIS MINIATA*

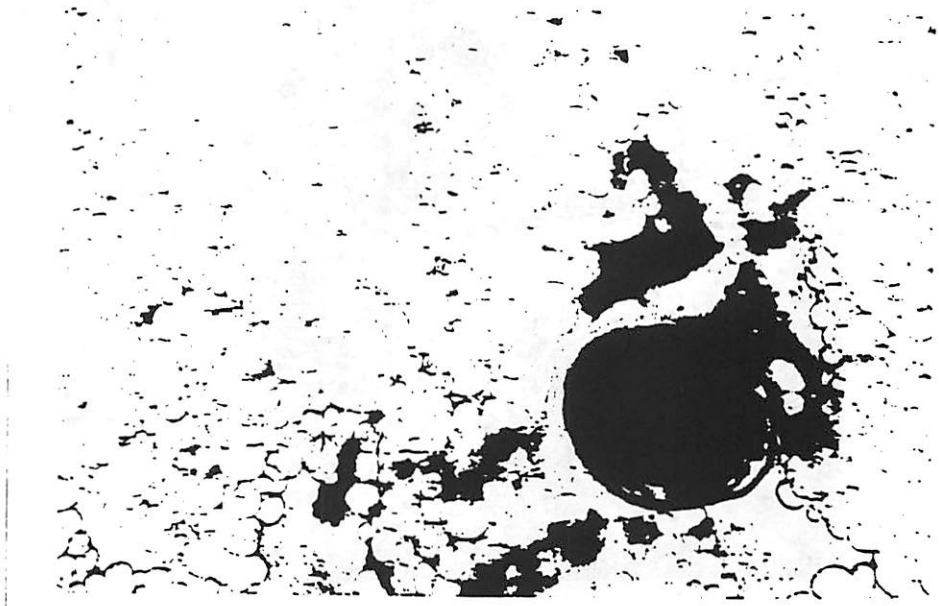


Plate 1. Wet mount of ovarian tissue from *C. miniata* caught July 15, 1993. 40x

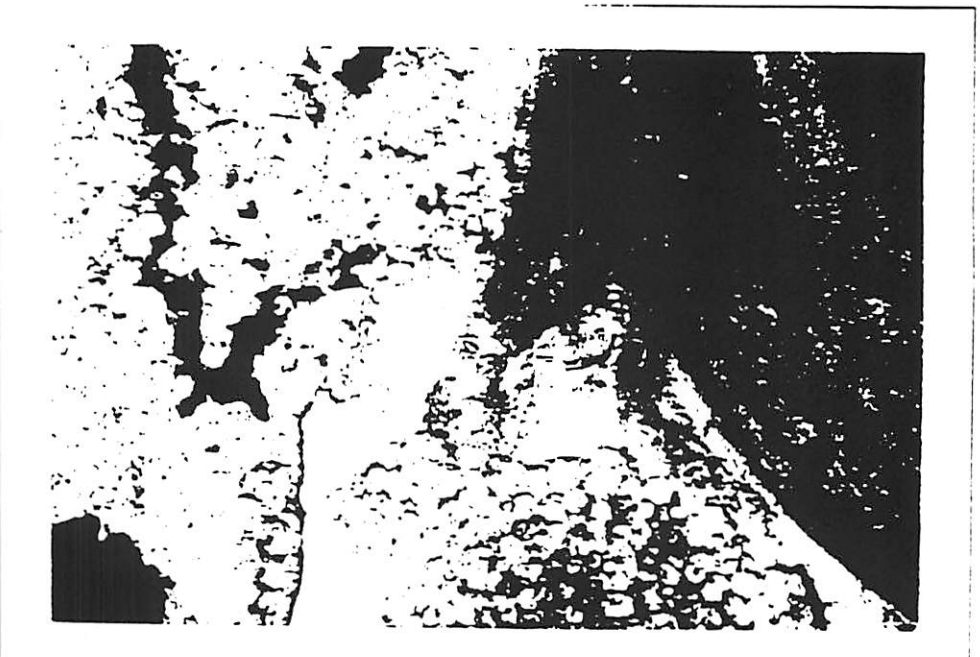


Plate 2. Wet mount of gonadal tissue from a "transitional male of *C. miniata*. 40 x.

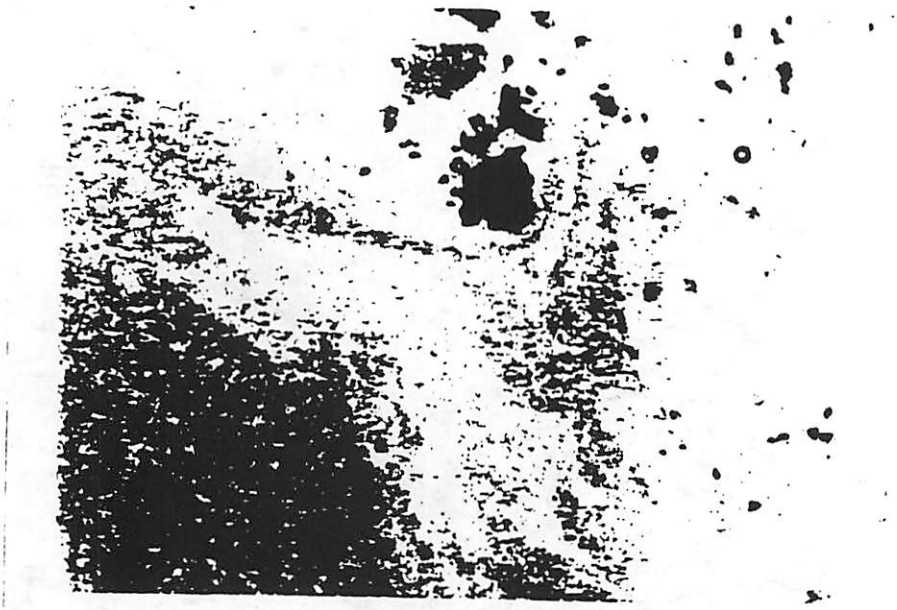


Plate 3. Wet mount of testicular tissue from *C. miniata* caught July 15, 1993. 40x.

APPENDIX 10
PROCEDURES FOR THE ARTIFICIAL PROPAGATION OF GROUPERS IN MICRONESIA

PROCEDURAL GUIDE

PROCEDURES FOR THE ARTIFICIAL PROPAGATION OF GROUPERS IN MICRONESIA

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LITERATURE CITED . . . . . .

INTRODUCTION

Groupers are a preferred species for fishermen because of their high market value in local and foreign markets as well as their suitability as a food source. Groupers are generally an easy catch in areas where overfishing has not depleted the stocks, because the fish are curious by nature and almost any bait will satisfy their voracious appetites (Myers, 1991). Efforts to conserve native stocks represents a loss of revenue and food on the table, and in several areas of Micronesia, specific grouper populations have all but disappeared. In Guam, for example, preferred species such as *Epinephelus fuscoguttatus*, *E. microdon*, and *Cephalopholis miniata* are a rare catch.

The artificial propagation of groupers represents a viable means to circumvent this dilemma by the generation of income from the sale of hatchery-produced juveniles, growout culture, and the augmentation of natural stocks via stock enhancement.

Development of grouper hatchery technology, in general, has been limited because of the biological peculiarities of groupers. Grouper hatchery technology in Micronesia is hindered by the lack of facilities at which to carry out hatchery production or, in the case of Guam, depleted stocks in surrounding waters where facilities exist.

PURPOSE

The intent of this manual is to provide the basic procedures for developing a hatchery technology for groupers. Much of the rationale for many of the procedures is based upon preliminary work on *Epinephelus microdon* and *E. fuscoguttatus* species performed for this consultancy. The reader is asked to refer to appropriate sections in the precursory Final Report and appendices for purposes of illustration. However, some of the procedures are similar to those employed for the culture of striped mullet (*Mugil cephalus*) and milkfish (*Chanos chanos*) and thus have been incorporated from Tamaru et al., (1993b).

This guide is geared for the recruitment of groupers from Palau and their propagation at the Guam Aquaculture Development and Training Center. It cannot be considered a complete hatchery manual, as many of the procedures are still being refined and require validation at commercial scale. This procedural guide does, however, provide a basis from which a complete technical manual can later be contrived.

ESTABLISHING A CAPTIVE BROODSTOCK

The first step in developing a hatchery technology for groupers is to obtain live adult individuals (i.e., 25-50) of the desired species. The number is dependent upon factors such as, the available holding facilities and manpower, the activity

objecti s (e,g,. res rch or production), projected hatchery outputs, and availabil_ y of feed.

Capture

Local fishermen in Palau have long known that various species of groupers are easiest to catch during annual spawning aggregations (Johannes, 1981). Spawning aggregations of "temekai" (e.g., *E. microdon*, *E. fuscoguttatus*), and "tiau" (e.g., *Plectropomus areolatus*, *P. leopardus*) take place in various passages throughout the barrier reefs known only by the fishermen (FitzGerald et al., 1991). Their knowledge extends from annual spawning occurrences to the times of the month (i.e., lunar cycles) and day (i.e., tides) when fishing is best. They also know in great detail what baits are best for a particular species. Therefore, it is highly advisable tc mploy the services of a local fisherman to aid in locating a des d species of grouper in the waters surrounding Palau.

One particular channel (Ngerum aol) has historically been a site of spawning aggregations and therefore heavily fished. Since 1980, the area has been declared a sanctuary to help protect against overfishing. Fishing for broodstock is permitted, however, national and state collecting permits are required.

A pictorial essay of the procedures for catching groupers and their subsequent treatment is presented in the Final Report, Appendix 1, Plates 1-5.

Materials Required:

1. boat with live bait well
2. hand lines (100 yards of 50-130 lb test), assorted hooks (size 20-36), and lead weights*
3. bait (e.g., squid, sardines, tuna, reef fish)*
4. bait knife and cutting board
5. large scoop net
6. 12 cc syringe with 20-gauge hypodermic needle
7. polyethylene cannula (0.86 mm i.d., 1.52 mm o.d.)
8. tape measure
9. holding tanks (1000 to 4000 liters)
10. seawater and aeration systems
11. Prefuran (Argent Chemical, Redmond, Washington, USA)

* = varies with the species is to be captured

Step 1. Determine the Target Species: A decision should be made prior to fishing as to what species is to be captured. This will determine what time of the year to fish and the fishing gear and bait to be used. For example, "tiau" aggregate in the channels between March and June and require moderate size fishing gear. "Temekai" aggregate between June and August and require larger fishing gear and bait (i.e., reef fish and/or tuna). *Cephalophilis miniata* can be caught

year-round inside of the barrier reef with smaller fishing gear and prefer sardines.

Step 2. Removal of Air From the Coelomic Cavity: Most species of groupers are caught in relatively deep water. When brought on board a boat their coelomic cavity will normally fill with air and swell to compensate for the decrease in pressure as they rise to the surface. After the captured individuals have been gently "unhooked" and placed in the bait well the air must be removed from the cavity of those floating upside down with a swollen abdomen. Hold the fish abdomen-side up with the head downward. Use the syringe to puncture the side of the abdomen as close to the anus as possible. Be sure to remove the plunger so that the air can escape. After the trapped air has escaped (i.e, one to two minutes), remove the syringe and release the fish back into the bait well. This activity can be repeated, if necessary.

Step 3. Return to Hatchery: When the desired number of individuals are obtained from the ocean, they should be taken to the hatchery as soon as possible. During the return trip, the fish should be checked frequently and the water in the bait well changed every 20 minutes or so. Groupers tend to regurgitate bait and sometimes the entire stomach contents after capture and when placed in the bait well. Remove any debris vomited by the captured individuals to prevent fouling of the water and reingestion.

Step 4. Back at the Hatchery: At the hatchery, place all captured individuals in holding tanks containing seawater and supplied with continuous aeration. Continue to remove air from the coelomic cavity of affected individuals as necessary. Allow broodstock to recuperate for at least 24 hours before handling again.

Step 5. Antibiotic Treatment: The captured fish will unavoidably receive bruises and/or abrasions from the capture process and should be treated at the hatchery with an antibiotic. The complete procedure for antibiotic treatment is presented in the section "First Aid" under Parasites and Disease. Captured individuals can be fed from the second day after being captured unless spawning or shipping activities must be undertaken immediately after recuperation from capture.

Air Transport of Live Fish

Establishing a grouper broodstock outside of Palau requires transportation by air to their final destination. The following sections describe the methods for preparing the broodstock for long distance transport.

Materials Required:

1. scoop nets
2. seawater and aeration systems
3. oxygen cylinder with two stage regulator
4. plastic buckets
5. 28" x 16" 3-ml in thickness plastic bags
6. strapping tape
7. rubber bands or electrical tape
8. 5-liter plastic pitcher
9. gang valve and airstones
10. ice
11. 100-liter plastic container
12. scale (50 kg capacity)
13. styrofoam shipping boxes
14. airline shipping bags

Step 1. Preparation for Transport: Preparation for transport can take several days. Transportation of live fish by air has restrictions imposed by the airlines. Before the shipping date, obtain all necessary documents (e.g., health certificate, agriculture inspection forms, certificate of origin, custom declarations, method of payment). If any one of these is not in order, the entire shipment can be detained upon departure or arrival at the destination. Obtain a flight schedule and make shipping reservations well in advance. The shipment can be sent as cargo on a domestic airline or handled by a freight forwarder. The time necessary for packing and travel to the airport should be considered. It is important that the individuals to be shipped **NOT BE FED** at least 48 hours prior to transport.

Step 2. Water Temperature: On the day of shipping, lower the water level of the tank containing the individuals to be shipped as much as possible. Be sure the tank continues to receive aeration. Place bags of ice directly into the water to lower the water temperature between 22 and 25°C. Fill a 100-l plastic container with seawater and lower to the same water temperature using bags of ice. The seawater in this container is used to fill the transport bags.

Step 3. Packing: All transport boxes must be clearly labelled with the address at final destination and the name and phone number of a contact person. Place two plastic bags (one inside of the other) into the styrofoam shipping box. Depending on the size of the individual(s) to be shipped, measure enough chilled water to completely cover the fish and place it into the transport bag. The entire transport box including its contents cannot exceed 45 lbs when shipped by domestic airline. Place the desired number of individuals into the transport box and fill the bags approximately 2/3 full with oxygen. Seal the bags with rubber bands or

electrical tape to insure a tight seal. Close the transport box and seal with strapping tape. Place the entire transport box into a large plastic bag obtained from the airlines and seal. Take boxes to the airport and ship. If using the services of a freight forwarder, the packing must be coordinated with the forwarding service pick-up time which is usually two to three hours before departure of the flight.

Step 5. Acclimation: When the fish have arrived at their final destination remove the fish from the boxes and leave them in the plastic bags. Float them in the receiving tanks for approximately ten minutes before emptying the fish and shipping water directly into the receiving tank. The receiving tank should be provided continuous aeration and the seawater exchanged at a rate of 100%/day. The transported fish should be observed in the receiving tank for at least 48 hours before removal to another tank. Food can be offered after the fish have acclimated. Small amounts should be offered and personnel should watch to see that it is consumed. Any uneaten food should be removed. UNEATEN FOOD SHOULD NEVER BE LEFT TO ACCUMULATE ON THE BOTTOM OF THE TANK for it will decompose and foul the water. The fouled water could result in bacterial growth on the fish which could be fatal without treatment.

REPRODUCTION

To understand the reproductive process in fishes and the rationale for techniques developed for controlling maturation and spawning, hatchery personnel should have some comprehension of the hypothalmo-pituitary-gonadal (HPG) axis. A schematic representation of the axis and the levels at which it is influenced by environmental and hormonal parameters is presented in Figure 1.

Broodstock Maintenance

The main task of the hatchery operator is to alleviate any stress that would prevent the captive broodstock from their normal reproductive processes. Excessive handling, overcrowding, diet, or changes in tank conditions (temperature, salinity pH, or dissolved oxygen) are stressors that, if not alleviated can inhibit growth and reproduction (Billard et al., 1981). Daily maintenance activities are monitoring water quality, feeding, tank maintenance and observation of the broodstock. These activities are construed as tedious but are essential for a healthy and productive broodstock.

▸ Tank Design

Natural spawning of groupers has been achieved in earthen bottom ponds, concrete tanks (100 m^3) and net cages (5x5x3 m) for

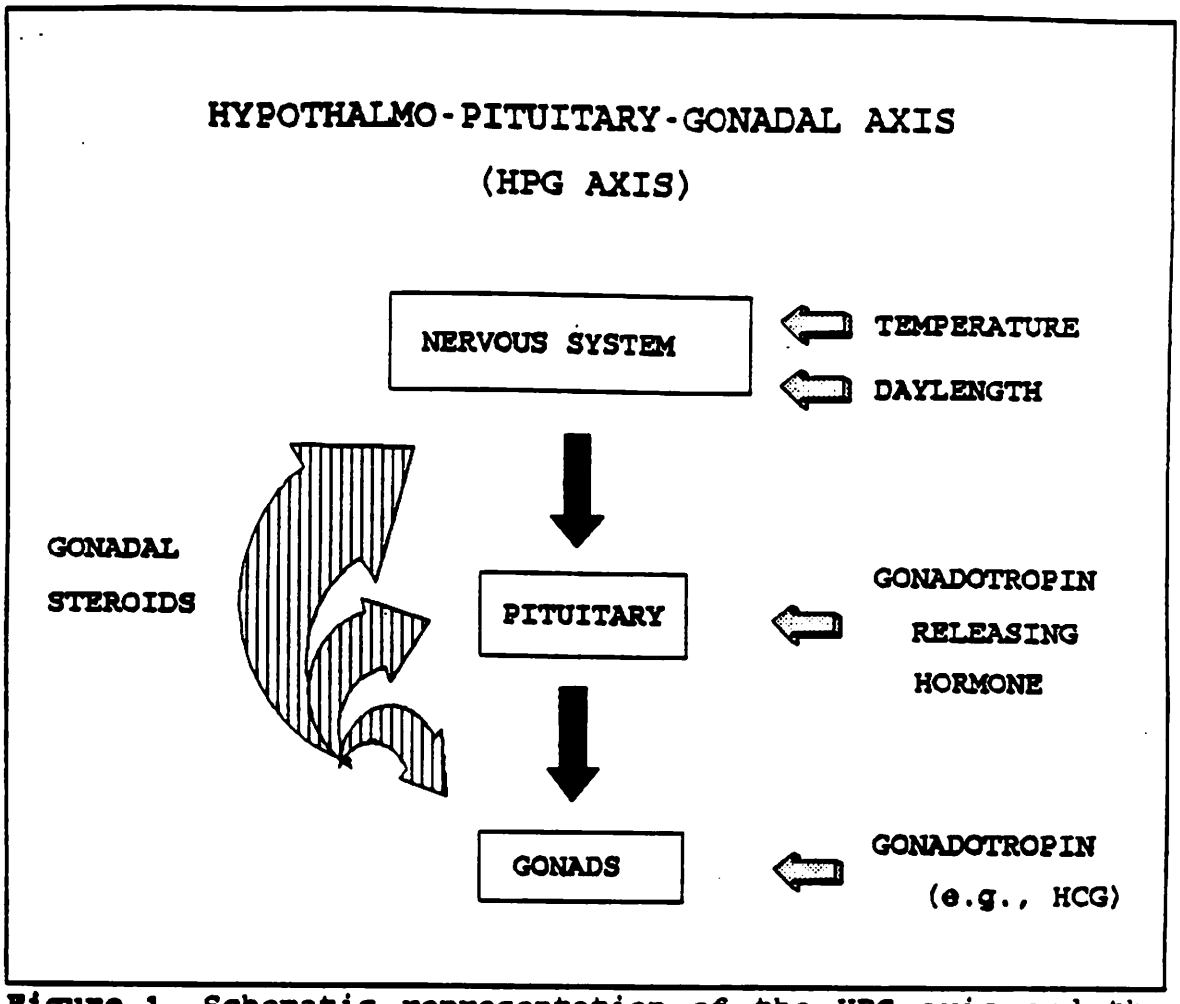

Figure 1. Schematic representation of the HPG axis and the levels of action for various hormones. From Tamaru et al., (1993b).

E. salmonoides, *E. fario* and *E. fuscoguttatus*, respectively (Tamaru et al., 1993a), when stocked at a 1:1 male/female ratio at a density of <- 1 fish/m^2. For the purposes of this procedural guide, the existing tanks at GADTC will be used as reference. Seawater entering into the tank should be filtered by gravel or a filter bag (10 µm size). The seawater system should have the capacity to achieve a sustained flow rate sufficient for a minimum of 100% water exchange/day. Since groupers are known to be territorial in the wild, the broodstock tank should contain hiding places, which can be made from PVC pipe or concrete blocks. To prevent excessive algal growth, the tanks should be covered with a shade cloth. Inordinate algal growth is conducive to the harboring and outbreak of several pathogens. It is common practice in mariculture to place herbivores into broodstock tanks to naturally crop the growth of macroalgae. However, because of the voracious carnivorous

feeding habits of groupers, large milkfish (i.e., 2-3 kg body weight) are recommended.

▸ Tank Maintenance

Siphoning of the tank bottom should be done on a regular basis to remove excess food and debris and to prevent the tank from becoming anaerobic. At least once a year the entire pond should be emptied and allowed to sun dry before use again.

▸ Water Quality

The most important factors affecting water quality are water temperature, dissolved oxygen, and salinity and they should be monitored daily. A thermometer (°C), dissolved oxygen (DO) probe and a refractometer are used to measure these parameters, respectively. The normal practice is to take daily measurements in the morning and afternoon. Readings should be logged in a manner that the data can be easily summarized and scrutinized for changes. Deviations outside of "normal" values require corrective action.

▸ Observation

An important part of the daily routine is to observe the broodstock for changes in appetite or behavior at the hatchery. This requires the personnel responsible for the care of the broodstock to be familiar with the swimming and feeding patterns of the fish. Loss of appetite and erratic swimming behavior are indications of the presence of parasites and/or poor water quality and if left unattended could result in a broodstock catastrophe.

▸ Feeding

At present, there is no formulated feed for use in grouper culture. Trash fish fed exclusively at a rate of 2-5% body weight/day and distributed between two or three separate feedings has resulted in natural spawning of captive broodstock in Taiwan and -Singapore (Tamaru et al., 1993a). The feed should be distributed as evenly as possible. As mentioned previously, groupers are extremely territorial and during feeding the larger males will hoard all of the food if presented in only one area of the tank. Time should be taken during the feeding process to insure that all individuals get food to reduce the probability of cannibalism (Figure 2).

Examining Broodstock

The monitoring of broodstock is an essential part in establishing the spawning season and assessing the general health of the fish. Broodstock examinations should take place at monthly intervals. It is recommended that all broodstock individuals be identified with a PIT tag (Identification Devices, Boulder,

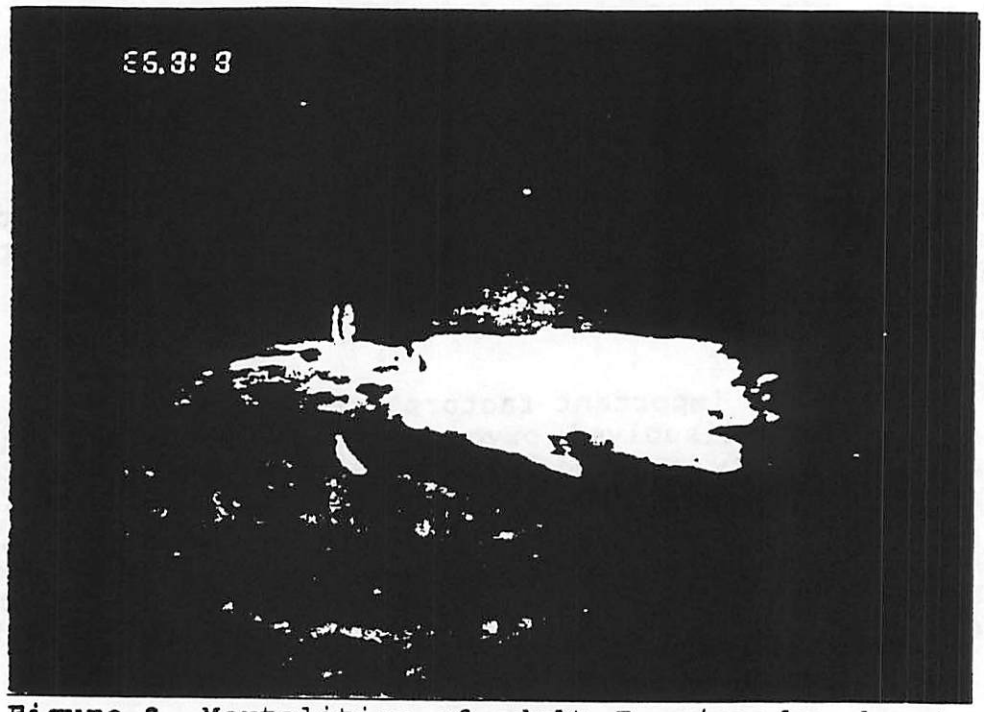

Figure 2. Mortalities of adult *E. microdon* due to cannibalism.

Colorado, USA). This particular tag is implanted into the dorsal musculature and when activated with a reader transmits a 10 digit code. This is a means of permanent identification to aid in the evaluation and monitoring of the broodstock.

Materials Required:

1. PIT tag reader and tags
2. scale (10 kg capacity)
3. measuring board
4. polyethylene cannula (0.86 mm i.d., 1.52 mm o.d.)
5. large scoop net
6. solution of 5% formalin in seawater
7. 10-ml sample vials with screw caps
8. 1000-liter holding tank
9. aeration and seawater

Step 1. Length and Weight Determination: Lower the volume of the broodstock holding tank. Groupers can then be individually caught with scoop nets. Obtain the identification number of each fish and place on the scale to determine the body weight. Remove the individual from the scale and place on a measuring board to obtain total length. Record the data on an appropriate data sheet.

Step 2. Gonadal Biopsy: Hold the individual upside down and insert a polyethylene cannula into the cloaca approximately 2-

3 inches into the body cavity. Apply suction to retrieve a sample of the gonads and place sample into a 5% formalin in seawater solution by blowing through the cannula. Label each sample vial with the date of biopsy and the tag number of the fish.

Step 3. Physical Examination: Each fish should be examined for the presence of external parasites, discoloration, bruises or abrasions. If any abnormalities are detected the individual should be placed in a separate tank for treatment. The treatment tank should have running seawater and continuous aeration. Repeat Steps 2 and 3 until all of the broodstock have been examined. If parasites are detected the entire broodstock must be treated, including the tank.

Step 4. Examination of Samples: Each gonadal sample should be viewed under a compound microscope equipped with an ocular micrometer and the state of maturity recorded for each individual examined. If vitellogenic oocytes are observed, the average oocyte diameter from a minimum of 50 oocytes should be obtained.

Step 5. Summarizing the Data: Temporal changes in maturation and growth should be summarized and plotted to determine the spawning season based upon the presence of vitellogenic oocytes and spermatozoa. This data can then be correlated to temperature and daylength to provide indicators as to what environmental cues are mediating the reproductive process in groupers. Oocyte growth data from individual females must also be summarized to obtain the rate of oocyte growth. This data is invaluable for anticipating when the females will reach the state of maturity at which induction of spawning can commence.

Induction of Spawning

The hormonal induction of final maturation and spawning for groupers has been successfully achieved with human chorionic gonadotropin (HCG). This gonadotropin acts directly on the ovary. Alternative hormones used in mariculture are gonadotropin releasing hormones and their more potent synthetic analogues. These stimulate the pituitary into releasing its own gonadotropin (Figure 1). In either case, the ovary must be in an appropriate state of maturity and this must be assessed with each fish prior to administering the hormonal treatment.

The procedure for induction of spawning is as follows:

Materials Required:
1. 1-cc tuberculin syringe
2. 23 gauge hypodermic needle
3. HCG in 10,000 IU/vials

4. physiological saline
5. polyethylene cannula (0.86 mm i.d., 1.52 mm o.d.)
6. large scoop net
7. scale (10 kg capacity)
8. 4000-liter tank
9. seawater and aeration systems
10. solution of 5% formalin in seawater
11. 10-ml sample vials with screw caps
12. compound microscope with ocular micrometer
13. depression slide
14. disposable pasteur pipettes with bulbs
15. egg collector
16. calculator
17. aquarium scoop net
18. hand ally

Ste . Obtaining an Ova an Biopsy: Restrain a female with a s p net and insert t polyethylene cannu a through the clo and into the ovic t approximately 2- inches. By appl ng suction an ovarian sample can be retrieved. Blow out the sample into a vial containing a solution of 5% formalin in seawater. Label each vial with the PIT tag number of the individual and the date of biopsy.

Step 2. Mean Oocyte Diameter: The mean oocyte diameter is determined by measuring the diameters of 50-100 oocytes with a compound microscope equipped with an ocular micrometer. The mean oocyte diameter serves as a measure of the maturity of the female. Only females found to possess a mean oocyte diameter >400 μm should be used in spawning trials.

Step 3. Assessing Testicular Maturation: The state of maturity of male individuals to be used in the spawning trials also must be assessed. This can be done by restraining the individual in a scoop net and applying slight pressure to the abdomen. If no milt is expressed, the cannula is used as described for females. If milt can be obtained by either method, the male can participate in spawning trials.

Step 4. Administering the Priming Injection: HCG is supplied in glass vials and in lyophilized form and must be dissolved in physiological (0.7%) saline prior to administration. Using a 1-cc tuberculin syringe capped with a 23 gauge hypodermic needle place 2 ml of physiological saline into the vial containing 10,000 IU of HCG. This will result in a solution that contains 5000 IU/ml = 5 IU/μl. Obtain the weight of the selected female or male and administer a priming injection that is equivalent to 700 and 500 IU/kg body weight for female and male, respectively. A good location to administer the hormone is three scale rows below the dorsal fin. The normal practice is to administer the hormone between 16:00 and 17:00

hours. Place the treated individuals into a 4000-liter spawning tank equipped with running seawater and continuous aeration.

Step 5. Administering the Resolving Injection: Twenty four hours later, the second (i.e., resolving injection) is administered to the female using the procedure described previously. In this case, however, the dosage of the second injection is 1400 IU/kg body weight. Males need not be given a second injection. Return the treated individuals to the spawning tank and setup a collecting net within the drainage system as pictured in the Final Report, Appendix 3, Plate 4.

Step 6. Spawning: It is preferable to let the female spawn the ovulated oocytes and the males fertilize the spawned eggs naturally. In the case of *E.microdon*, this occurred approximately 8-12 hours after administration of the second injection. Spawned eggs are then collected using an egg collector (in the Final Report, Appendix 3, Plate 4) or by scooping the eggs with a fine mesh aquarium scoop net. Place the collected eggs into a 100-liter polycarbonate tank filled with seawater and aerate.

Step 7. Quantify the Number of Spawned Eggs and Percent Fertilization: Obtain a sample of eggs from the 100-liter polycarbonate tank using a 100-ml beaker and count the number of eggs/100 ml. Repeat this three times to determine the average number of eggs/100 ml and extrapolate to 100 liters to determine the total number of spawned eggs. From a random sample of approximately 100 eggs, determine the percent fertilization using a compound microscope. Fertilized eggs can be distinguished from unfertilized eggs by their cleaving cells (Final Report, Appendix 4, Plates E-H). Using the average number of eggs/100 ml, the appropriate number of eggs can be removed volumetrically.

Strip Spawning

Strip spawning is a method used to produce fertilized eggs in the event that spawning does not occur naturally. It is not a preferred method because of the physical strain placed on both males and females, as well as the inconsistent results regarding fertilization. The inconsistency results because of the variability in synchronizing the mixing of ovulated oocytes with milt. It is, however, a means of producing fertilized eggs that should be part of the hatchery technology. A pictorial essay of this procedure is found in the Final Report, Appendix 2.

Materials Required:
1. 25-liter plastic basin
2. 5-liter plastic pitcher

3. 20-liter plastic bucket
4. sea salt
5. refractometer
6. seawater and aeration systems
7. 12-cc syringe
8. polyethylene cannula
9. large scoop net
10. paper towels
11. 1-liter glass beaker
12. 100-liter polycarbonate tank
13. compound microscope
14. depression slide
15. hand tally
16. filter bag (10 μm)

Step 1. Obtaining Milt From Males: Catch a hormone treated male with the scoop net and hold the individual upsi down. Use a paper towel to wipe the cloaca dry. Appl; slight pressure to the abdomen and milt will be extruded from the cloaca. As the milt is extruded use a 12-cc syringe to collect approximately 5-10 ml of milt. Cap the syringe with a hypodermic needle.

Step 2. Staging the Female: During final maturation the oocytes will undergo ovulation and hydration and the abdomen of the female will noticeably swell (Final Report, Appendix 3, Plates 1 and 2). Approximately 12 hours after the second injection, an ovarian biopsy can be performed as described previously. When a female has completely ovulated, her oocytes can be stripped. An example of an ovulated oocyte is presented in the Final Report, Appendix 4, Plate C. A quick and simple means of determining whether the eggs have ovulated is to hold the cannula containing the ovarian biopsy up to a light source. Ovulated oocytes will have the appearance of a string of glass beads.

Step 3. Stripping and Fertilizing Eggs: When the female has completed ovulated hold her firmly above a dry plastic basin and apply pressure to her abdomen (Final Report, Appendix 2, Step 1). You may need to push several times to remove the majority of eggs. When the majority of eggs have been removed the female can be returned to the holding tank. Disperse the milt from the syringe over the stripped eggs (Final Report, Appendix 2, Step 2). Gently mix the eggs and milt with your hand (Final Report, Appendix 2, Step 3). Gently pour filtered seawater (35 ppt) from the 5-liter pitcher into the plastic basin to activate the sperm, and allow fertilization to take place. Slowly fill the basin with seawater using the 20-liter bucket and gently mix the eggs and seawater with your hand (Final Report, Appendix 2, Step 4 and 5). Approximately ten minutes after adding the seawater the fertilized eggs will

begin to float to the surface and can be removed by skimming with the 1-liter glass beaker (Final Report, Appendix 2, Step 6). Place the collected eggs into a 100-liter polycarbonate tank filled with filtered seawater and aerate.

Step 4. Quantifying the Number of Spawned Eggs and Percent Fertilization: Same as described in Steps 6 and 7, respectively, in the previous section.

Incubation and Hatching

Incubation period extends from the time of fertilization until hatching. This time interval for *E. microdon* and *E. fuscoguttatus* is approximately 18 1/2 hours at 27°C. The main activity during this period is the development of the grouper embryos. The time frame and stages of ontogeny are presented in Table 9 and Appendix 4, Plates D-P, respectively, of the Final Report.

Materials Required:

1. 1000-liter fiberglass tank equipped with center standpipe
2. 250 µm nytex screened cap
3. aeration and seawater systems
4. 10 µm filter bags

Step 1. Stocking of Spawned Eggs: The incubation tank should be filled with filtered seawater (35 ppt) and provided with continuous aeration. The center standpipe should be capped with the 250 µm nytex screen and seawater provided at an exchange rate of 100%/day. Remove the appropriate volume from the 100-liter polycarbonate tank to result in 5×10^3 eggs and place them into the incubation tank. Allow the eggs to develop until they are ready to be stocked (i.e., somite stage, Final Report, Appendix 4, Plate N).

Step 2. Separation of Eggs: During the incubation process unfertilized or non-developing eggs (Final Report, Appendix 4, Plates Q and R) will turn opaque and become negatively buoyant at salinities between 35-40 ppt. When the embryos have reached somite stage, stop the flow of water and aeration. Allow the contents in the incubation tank to stand for approximately 15-20 minutes at which time the developing eggs will float to the surface. Skim the floating eggs and place them into the 100-liter polycarbonate tank.

Step 3. Quantifying the Eggs: When the majority of floating eggs have been obtained, fill the 100-liter polycarbonate tank with seawater till the 100-liter mark and obtain the total number and density of grouper eggs as described previously.

Step 4. Stocking the Eggs: The eggs in the polycarbonate tank are now ready for stocking into larval-rearing tanks. The desired number of eggs for each rearing tank can be removed volumetrically.

Egg Transport

Transportation of fertilized eggs is normally not part of hatchery operations since the broodstock and larval-rearing facilities are usually at the same location. However, during the initial phases of any hatchery development, the locale where broodstock are accessible and the location of larval-rearing facilities may be different, as is the case with the grouper work currently ongoing at MMDC and GADTC. Thus, until a captive broodstock is established, larval-rearing activities (at GADTC) are dependent on the transport of fertilized eggs from the site where spawning can be achieved (at MMDC).

When the hatchery technology for grouper is fully developed, shipment of eggs from an "egg production center" to various hatcheries may become an actual scenario as seen with the hatchery production of milkfish in Taiwan. Therefore a procedural guide for the transport of fertilized eggs is warranted.

Materials Required:

1. oxygen bottle with two stage regulator
2. styrofoam transport boxes and plastic bags
3. sea salt
4. 100-liter plastic trash can
5. fine mesh aquarium net
6. 5-liter plastic pitcher
7. refractometer
8. hand tally
9. strapping tape
10. electrical tape
11. 100-ml beaker
12. filter bag (10 m)

Step 1. Adjusting Salinity of Water for Transport: The transport of fertilized eggs at a salinity of 40 ppt aids in keeping the eggs suspended in the water column. A supply of 40 ppt seawater solution is prepared in a 100-liter container by adding sea salt to filtered seawater. Dissolve the sea salt and measure the salinity with a refractometer until the desired salinity is obtained.

Step 2. Stocking of Eggs: A specific number of eggs to be shipped should be determined. Place a corresponding volume of 40 ppt seawater into plastic transport bags. The size of the bags and volumes will vary depending on the quantity of eggs to be shipped. ***NOTE: TRANSPORT BOXES AND THEIR CONTENTS MUST NOT EXCEED 45 LBS IF SENT BY AIR.*** After obtaining the egg

density as described in the previous section, the number of eggs required to achieve a stocking density of 10-20 x 10^3/l can be removed volumetrically. These are then sieved through a fine mesh aquarium net and the eggs are then placed into each transport bag.

Step 3. Packaging of Eggs: Each of the plastic bags is then filled with oxygen and sealed with rubberbands (Final Report, Appendix 1, Plate 13). If transport is to take place by air it is advisable to use electrical tape to insure a tight seal. Place all bags into transport boxes and seal with strapping tape.

Step 4. Acclimation: When the eggs have arrived at the hatchery, float the unopened bags containing the eggs in the receiving tanks to allow the eggs to acclimate. After approximately 15 minutes open the bags and place the entire contents directly into the tanks.

PRODUCTION OF LIVE FEEDS

Production of live feeds is a major activity for the larval culture of any species and must be coordinated with the availability of fertilized eggs. Live feeds production for grouper larvae includes the culture of phytoplankton and rotifers, the hatching and enrichment of *Artemia* nauplii, the harvesting of copepods and the processing of oyster trochophores.

Phytoplankton Culture

Nannochloropsis oculata is the algal species recommended for use in the larval rearing of grouper. It is used primarily as a feed for culturing rotifers, one of the initial feeds of grouper larvae. However, its presence in the rearing tanks during larval culture of striped mullet has also been shown to be beneficial to overall growth and survival (Tamaru et al., 1993b). The main reason that this particular algal species is used in rearing fish larvae, in general, is its nutritional value (Watanabe et al., 1983). In particular, this species has been shown to contain elevated levels of long chain polyunsaturated fatty acids (PUFAs) which are essential for a large number of fishes. It has also been demonstrated that the nutritional quality of the algae fed to the rotifers directly effects the nutritional value of the rotifers (Tamaru et al., 1991).

The culture of this species of algae is carried out through a series of batch cultures where each series represents an amplification of its volume. A schematic representation of the process is presented in Figure 3.

The production of cultures indoors progresses through three stages: 1) stock culture, 2) flask culture, and 3) carboy and/or cylinder culture. In the indoor production scheme, the flask and

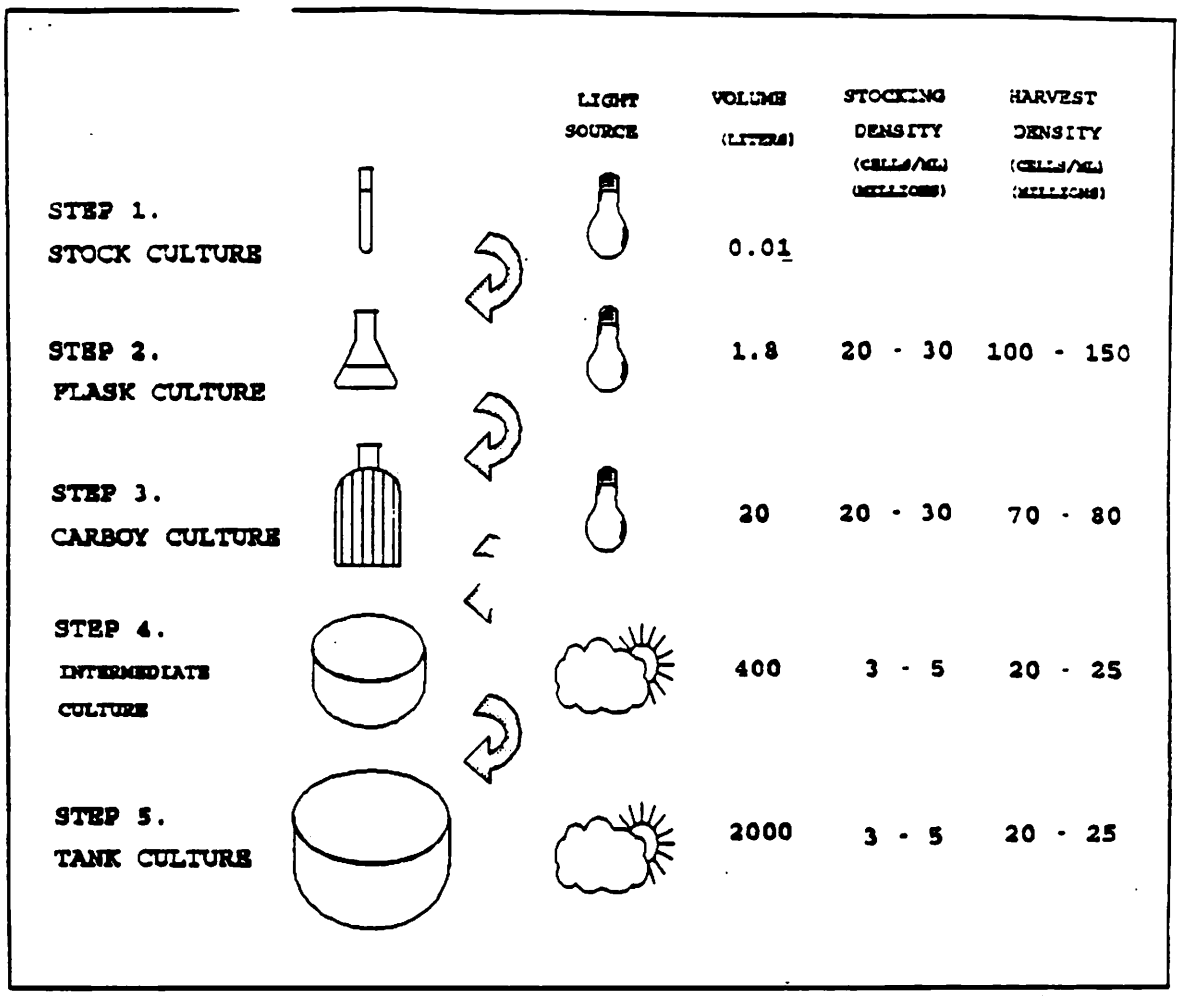

Figure 3. Flow chart of the process for production of phytoplankton. From Tamaru et al., (1993b).

carboy cultures represent starting and intermediate stages in the culture of algae, respectively. Carboy and cylinder cultures are used as inocula for outdoor cultures.

Outdoor production of algae consists of two stages: 1) 400-liter tanks which are inoculated from carboys or a cylinder from the indoor algae room, and 2) 2000-liter tanks which are inoculated from the 400-liter tanks after they attain harvest density. At full production, two of the second stage (i.e., 2000-liter) culture tanks are required each day to support rotifer culture and larval rearing activities. The procedures for production and monitoring of indoor cultures have been described in detail in Tamaru et al., (1993b). The procedure for outdoor cultures is as follows:

Materials Required:

1. six x 400-liter fiberglass tanks
2. 14 x 2000-liter fiberglass tanks
3. aeration system
4. two submersible pumps

5. garden hoses
6. top loading balance (10 kg capacity)
7. 20-liter plastic buckets
8. compound microscope
9. hemacytometer
10. hand tally or multicell counter
11. chemicals (i.e., nutrients, sodium hypochlorite, sodium thiosulfate)
12. pipettes
13. storage room

Step 1. Intermediate Culture Preparation: A 400-liter starter culture tank should be chlorinated between use and scrubbed clean. Fill with filtered water at a salinity of 25-28 ppt and chlorinate with a 5.25% sodium hypochlorite solution. Commercial bleach solutions, such as Clorox or Purex, are generally available as 5.25% sodium hypochlorite solutions. Aerate for 24 hours. Dechlorinate with the addition of sodium thiosulfate. The proportions of hypochlorite and sodium thiosulfate needed for various tank sizes are presented in Table 1.

Table 1. Amounts of hypochlorite and sodium thiosulfate for various tanks sizes.

TANK VOLUME (liters)	5.25% NaOCL SOLUTION (ml)	SODIUM THIOSULFATE (grams)
500	1500	75
1000	3000	150
2000	6000	300
5000	15000	750

Step 2. Intermediate Culture: Nutrients (e.g., Mass Pack, Florida Aqua Farms Inc., Florida, USA) can then be added according to the instructions with an inoculum from the algae room (20-liter carboy). After addition of the inoculum, the initial stocking density should be 3 to 5 x 10^6 cells/ml. Monitor cell growth, and use as an inoculum for the production cultures when densities of 20 to 25 x 10^6 cells/ml are attained.

Step 3. Production Cultures: Culture tanks should be scrubbed clean and rinsed between each use. Tanks holding the final production stage for algae (i.e., ≥ 2000 liters) **DO NOT** need to dechlorinated. Fill tanks with water at a salinity of 25-28 ppt and inoculate with phytoplankton from the intermediate

tanks to obtain an initial density of 3-5 x 10^6 cells/ml. Nutrify as described previously and monitor cell growth.

Step 4. Harvest: When cell densities reach 20 to 25 x 10^6 cells/ml, the culture is ready to be used as food for rotifer culture or as background algae for use in the larval rearing tanks. When the culture is completely harvested the process is started again.

Rotifer Culture

The rotifer *Brachionus plicatilis* has been recognized as pivotal for successful culture of a variety of marine fish larvae (Lubzens, 1987; Lubzens et al., 1989). Rotifers are small (i.e., 140-230 µm) multicelled organisms that filter food particles out of the water column by means of a ciliated corona located on the anterior portion of their body. The main reasons for its popularity are its small size, tolerance to a wide range of salinities, fast reproduction rate, ability to be cultured at very high densities (i.e., >1000 rotifers/ml), slow swimming speed, and tendency to stay suspended in the water column (Fulks and Main, 1991).

Rotifers can be grown on a variety of feeds, but the most common practice is the use of phytoplankton cultures. As stated previously, the recommended phytoplankton is *N. oculata* because of its higher fat content. The method for its culture is as follows:

Materials Required:

1. six 1000-liter fiberglass tanks equipped with center drain
2. 60 µm nytex bag
3. 100-liter plastic basin
4. dissecting microscope well counting dish
6. Veeder-Root multicell counter (5 well)
7. 1-ml rotifer counting pipette
8. magnifying eyepiece (10 x)
9. data sheets
10. Lugol's solution
11. airstones and airline tubing
12. 500-ml graduate cylinder
13. 3-liter graduate pitcher
14. 20-liter plastic buckets (marked with 1-liter increments)
15. calculator
16. *Nannochloropsis oculata*
17. freshwater source
18. seawater source
19. refractometer

All rotifer culture tanks should be elevated about three feet above the ground or near a drainage ditch in which a collection net can be placed. This simplifies collection of rotifers from the tanks which is done by draining the culture through a collecting

net. The method described assumes enough rotifers are available to serve as an inoculum. Each step is schematically presented in Figure 4.

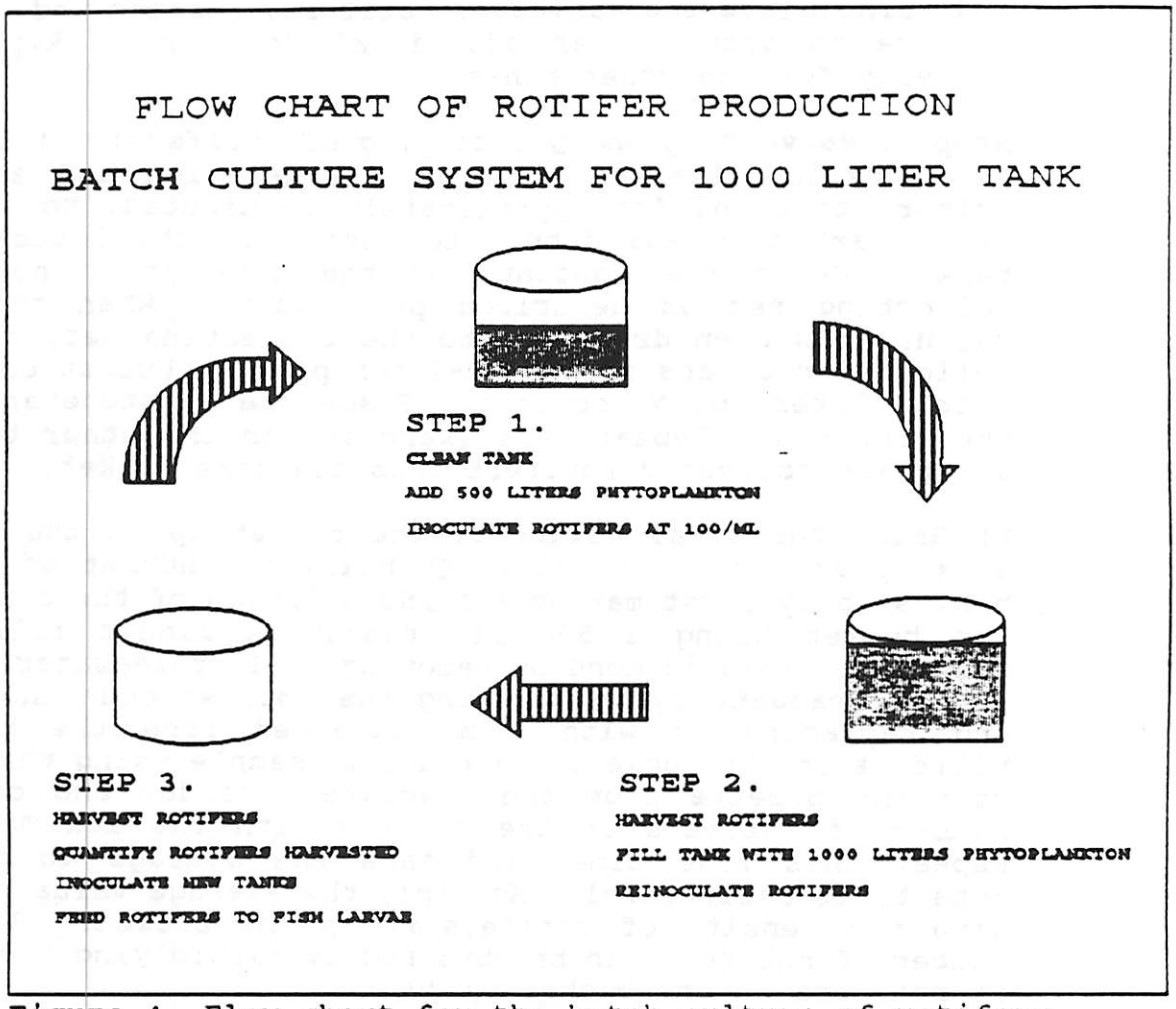

Figure 4. Flow chart for the batch culture of rotifers.

Step 1. Tank Preparation and Inoculation: A) Clean the inside of three 1000-liter tanks by scrubbing with a scrub brush and hosing with fresh water. Add 500 liters of *N. oculata* ($\geq 10 \times 10^6$ cells/ml) from the outside phytoplankton cultures into each of the three tanks. Check the salinity with a refractometer and adjust salinity with fresh water or more phytoplankton culture to attain a salinity ranging between 20 and 28 ppt. Inoculate the three tanks with enough rotifers to achieve an initial density of 100 ind./ml. Aerate the tanks and allow the culture to grow for 24 hours.

Step 2. Second Day: After 24 hours, turn off the aeration to the tanks and allow the contents to remain undisturbed for approximately 15 minutes. Harvest all of the rotifers from a

single tank by draining its contents through the 60 μm nytex collection net. Be sure that the net is suspended in a plastic tub filled with seawater to lessen the shock of harvesting. Fill the tank with 1000 liters of phytoplankton and reinoculate the harvested rotifers. Aerate and allow the culture to grow for an additional 24 hours. Repeat this activity for the other tanks.

Step 3. Harvesting and Quantifying of Rotifers: A) After the 24 hours has elapsed turn of the aeration and allow the culture to stand for approximately 15 minutes, to allow the large particles and debris to settle to the bottom of the tank. Drain the contents of the tank into the rotifer collecting net as described previously. When the entire culture has been drained into the collecting net, place the collected rotifers into a 20-liter plastic bucket containing 3 to 5 liters of *N. oculata*. Place one airstone and aerate the contents. Repeat this exercise for the other two tanks and place collected rotifers into the same bucket.

B) Bring the total volume of the bucket up to the 20 liter mark by adding *N. oculata*. Quantify the number of rotifers harvested by first making a 1:250 dilution of the contents in the bucket using a 500 ml graduate cylinder filled with seawater. This is done by removing 2 ml of seawater from the 500 ml graduate cylinder using the rotifer counting pipette and replacing it with 2 ml obtained from the harvested rotifers in the bucket. Take a 1 ml sample using the rotifer counting pipette from the graduate cylinder and count the number of rotifers in the pipette with the 10x magnifier. Repeat this five times and take the average to give the density of rotifers/ml. Multiply the average value by 250 to give the density of rotifers/ml in the bucket. The total number of rotifers can be obtained by multiplying the density of rotifers in the bucket by 20,000.

C) Repeat Step 1 for all of the tanks and inoculate by removing the appropriate volume from the bucket to result in a stocking density of 100 rotifers/ml. The remaining rotifers are available as food for the grouper larvae. Be sure to keep the rotifers aerated before stocking into the larval rearing tanks.

The cycle from inoculation to harvest for three tanks takes place over a 48-hour period. A continuous daily production of rotifers is achieved by treating a second set of three tanks in the same manner, however, the inoculation step is offset by one day from the other set of three tanks. Thus, on any given day three tanks can be harvested and the other set of three tanks are midway to being harvested.

Harvesting of Copepods

GADTC is fortunate in that the large shrimp broodstock raceways contain a considerable number of copepods. The only drawback is that their availability is affected considerably with the normal tank maintenance for the shrimp broodstock. The collection of copepods is most efficient during the night when their phototactic characteristic is an advantage for their harvest.

Materials Required:

1. 60 μm collecting net
2. plastic tub
3. 1 inch I.D. siphon hose
4. flashlight
5. medium mesh aquarium net
6. 5-liter pitcher
7. dissecting scope
8. Lugol's solution
9. 10-well depression slide
10. hand tally
11. seawater and aeration systems

Step 1. Harvesting of Copepods: Turn off the aeration system to the broodstock tanks and suspend a flashlight above the surface of the water. Place one end of the siphon hose in the water where the beam of light penetrates the surface. Place the other end of the siphon hose where the effluent will flow through a medium meshed (500 μm) aquarium net and finally through a 60 μm nytex collecting net. The collecting net should be suspended in a plastic tub filled with seawater. After the copepods have been harvested be sure to turn on the aeration system for the shrimp broodstock tanks.

Step 2. Quantifying the Harvested Copepods: Concentrate the collected copepods in the collecting net and place into a 5-liter plastic pitcher. Fill the pitcher to the 5 liter mark. Aerate the contents of the pitcher and retrieve a 10-ml aliquot. Place the 10-ml sample into a watch glass and add one to two drops of Lugol's solution. Gently rotate the watch glass to mix the Lugol's solution, inactivate the copepods, and concentrate them into the center of the watch glass. Using a disposable pasteur pipette, distribute the concentrated copepods into a 10-well depression slide, place under a dissecting microscope and count the number of copepods in each well. Continue until all of the copepods are counted. Repeat this activity three times and obtain the average number of copepods/10 ml. Extrapolate (i.e., multiply by 5000) to obtain the total number in the 5-liter pitcher. The copepods can then be stocked into their respective rearing tank by removing the appropriate volume.

Oyster Trochophores

Oyster trochophores can be used as an initial feed for grouper larvae and are purchased from MTL Biotech Ltd., Vancouver, B.C., Canada. The trochophores are cryogenetically preserved and need to be reactivated prior to use as feed. The trochophores are shipped prepackaged in plastic straws in a "dry shipper" containing liquid nitrogen. The straws containing the trochophores can be held in the shipping container for at least a week. The straws are then removed as needed and the trochophores are rejuvenated. The procedure is presented in the Final Report, Appendix 6.

Artemia Nauplii

It was recognized very early by a number of investigators that freshly hatched *Artemia* nauplii are a high value feed for fish fry. Because of their size, *Artemia* nauplii also represent, in many cases, the only available food source for the early stages of fish and crustacean larvae (Bardach et al., 1972). Although critical to larval culture, they have some drawbacks. The first is that the dry weight and caloric content of nauplii can decrease as much as 25% within 24 hours after hatching when kept at 25°C. This means the nauplii must be fed to the larvae as soon as possible after hatching or be stored at low temperatures to decrease their rate of metabolism. The second is that they have been found to be nutritionally deficient, especially in the long chain PUFAs. This shortcoming, however, can be corrected by enriching the *Artemia* nauplii.

▸ Hatching of *Artemia*

Artemia cysts can be purchased from a variety of sources however, the majority (i.e., 80%) of the cysts that are sold commercially originate from the Great Salt Lakes in Utah, U.S.A. (Sorgeloos et al., 1991). The operator should be aware that there is considerable variability in the hatching percentage of cysts from different origins as well as from different batches from the same strain (Sorgeloos et al., 1986). A check of the hatching percentage should be conducted periodically. This is done by incubating a known amount of cysts (i.e., 1 gram of cysts equals approximately 250,000 cysts) and hatching them. By estimating the total number of nauplii and dividing by the number of cysts incubated, the hatching percentage can be calculated.

The cysts are packaged in vacuum packed cans that must be refrigerated after opening. Hatching of *Artemia* is a relatively simple process but there are some parameters that should be followed. A constant water temperature (25 to 28°C), salinity (15 to 35 ppt), pH ≥ 8.0, saturated DO levels accomplished by vigorous aeration, and strong illumination (2,000 lux) are essential to initiate the hatching response (Sorgeloos et al., 1986).

Materials Required:

1. two 100-liter fiberglass cylinders with funnel shaped opaque bottom and drain
2. 50-liter glass aquarium
3. black plastic sheet
4. 12" glass tubing (1/8" diameter).
5. 10' airline tubing (1/8" diameter)
6. flashlight
7. 20-liter plastic buckets marked with 1 liter graduations
8. aeration and seawater system
9. top loading balance (10 kg capacity)
10. submersible heaters
11. collection net 100 μm*
12. 100-liter plastic basin
13. tygon tubing 1" diameter
14. 500-liter graduate cylinder
15. 1-ml glass pipette
16. 10x ocular magnifier
17. lights (100 watt)

* = rotifer collecting net may be substituted

Step 1. Incubation of Cysts: Fill fiberglass cylinders with seawater and aerate vigorously. Place heaters in cylinders and set temperature at 28°C. Measure amount (grams) of *Artemia* cysts required and add to the cylinders. (This is assuming that the hatching percentage of the *Artemia* cysts is already known). An alternative method to measuring cysts by weight is to measure them by volume. Convert # of grams of cysts = # of ml of cysts. Aerate the cysts vigorously and turn on the lights above cylinders and illuminate. Incubate overnight.

Step 2. Separation of Empty Cysts and Nauplii: After *Artemia* have hatched (at this stage the *Artemia* are called nauplii) turn off aeration and remove heater and airstone. Illuminate the bottom of the tank with a strong light. Wait approximately 10 minutes for the empty cysts to float to the surface of the water. Support collecting net in 100-liter plastic tub securely and fill tub with seawater. Separate the cysts from the hatched *Artemia* by draining the cylinder into the collecting net. Be sure to close the drain before the cysts are emptied.

Step 3. Collection of Nauplii: Fill aquarium with seawater leaving enough room to accommodate the collected nauplii. Remove the collecting net from the plastic tub and rinse collected nauplii with running seawater and place contents of the collecting net into the aquarium. Do not aerate. Cover the aquarium with a black plastic sheet leaving a space at the bottom. Turn on flashlight and illuminate the bottom of the aquarium. Wait approximately five minutes and siphon nauplii

from the bottom of the aquarium using the glass tubing connected with airline tubing as a siphon hose. Let the nauplii drain into a 20-liter plastic bucket. *CAUTION: DO NOT LET NAUPLII COLLECT ON THE BOTTOM OF THE AQUARIUM FOR LONGER THAN 15 MINUTES.*

Step 4. Quantifying Nauplii Harvested: Bring the volume of the siphoned nauplii in the plastic bucket up to the 20 liter mark and aerate vigorously. Fill graduate cylinder with seawater to the 500 ml mark. Remove 2 ml of seawater with the 1-ml pipette and replace with 2 ml of nauplii from the plastic bucket. Make sure that the nauplii are aerated (i.e., mixed) vigorously. Mix the contents of the graduate cylinder and remove 1 ml aliquot with the pipette and count the number of nauplii in the pipette using the 10x magnifier. Repeat five times to determine the average number of nauplii per ml. Multiply this value by 250 to estimate the number of nauplii/ml in the plastic bucket. Multiply this number by 20,000 ml to determine the total number of nauplii available. The amount of nauplii required for feeding or for enrichment can be removed volumetrically.

▸ Enrichment of *Artemia*

Most strains of *Artemia* are lacking in some of the nutritional elements required by certain species of fish, in particular the long chain omega-3 polyunsaturated fatty acids (PUFAs) as presented by Sorgeloos et al., (1991). This deficiency, however, can be surmounted by an "enrichment" process in which the *Artemia* nauplii are fed a source rich in PUFAs to improve their nutritional composition. The enrichment procedure for *Artemia* nauplii using Protein Selco is presented below.

Materials Required:

1. blender
2. 20-liter bucket
3. Protein Selco (Artemia Systems, Belgium)
4. *Artemia* nauplii
5. 200 µm collecting net
6. seawater and aeration system

Step 1. Obtain Artemia Nauplii: A rough estimate of the amount of nauplii required for the following days feeding should be obtained. Place the nauplii into the bucket with seawater at a density of 300-400/ml. To insure that the total days ration of nauplii is enriched, you may need to increase the volume or number of buckets to be used in the enrichment process. Aerate the nauplii vigorously.

Step 2. Preparation of Protein Selco: Weigh out the appropriate amount of Protein Selco required to result in a

300 ppm final concentration in the enrichment container. Place the Protein Selco into the blender and add fresh water. Be sure to take into account the volume of freshwater being used in mixing. Blend the mixture until it has a creamy appearance and add the blended mixture to the *Artemia* nauplii. Incubate the nauplii with the enrichment media overnight.

Step 3. Collection of Enriched Nauplii: The following morning collect the enriched nauplii with the collecting net and rinse with seawater. Place collected nauplii into a 20-liter bucket filled with seawater and aerate. Quantify the density of nauplii and remove the appropriate amount for feeding to the larvae.

Step 4. Storage of Enriched Nauplii: Enrich and process a large volume of nauplii as described. These can then be stored at 5°C and used for up to a week. The nauplii must be aerated during the storage process.

LARVAL REARING

Larval rearing is viewed as the most difficult aspect in developing a technology for the artificial propagation of grouper. The major reason is the small size of grouper larvae. Conventional methods such as using rotifers as an initial feed have been found to be ineffective and alternative sources (e.g., oyster trochophores, mussel larvae, and sea urchin larvae) have been employed to bridge the period from first feeding until the larvae are large enough to accept rotifers (i.e., 7-10 days posthatching). A second major impediment has been the reported cannibalism that occurs between 25 and 35 days posthatching.

At present there are two methods (extensive and intensive) for the rearing of grouper larvae. The extensive system is characterized by being conducted outdoors and in relatively large tanks or even ponds. Stocking densities are low (1-10 larvae/l) and the feed for the larvae is dependent on the productivity of the ponds. Results are highly variable, as expected for large-scale cultures occurring outdoors. However, the extensive method has one major advantage in that it does not require as much labor. The intensive system differs in that rearing is done indoors and in relatively smaller tanks (3-10 x 10^3 liters) stocked with high densities of larvae (30-40 larvae/l). The advantages are that the system is much easier to manage and the results are more consistent. However, it is labor intensive, especially during the period where cannibalism occurs and larvae have to be continually sorted.

Tank Preparation

As stated previously, one of the major differences between the intensive and extensive methods is the tank size and that the

extensive culture system is dependent on the productivity of the tank. The preparation of rearing tanks is presented below.

▸ Intensive Larval Rearing

Materials Required:

1. 2000 to 10,000-liter round fiberglass larval rearing tanks
2. 2" center drain with stand pipe and coupling
3. 250 µm nytex screened drain cap*
4. 550 µm nytex screened drain cap*
5. 12" inner diameter ring siphon drain (250 µm mesh)*
6. 12" inner diameter ring siphon drain (550 µm mesh)*
7. blower with aeration system
8. freshwater and seawater supply
9. 5' of 1/8" I.D. glass tubing
10. 10' of 1/8 " airline tubing
11. 5' of 1/4 " I.D. glass tubing
12. 10' of 5/16" tygon tubing
13. 10' of 1" I.D. tygon tubing
14. airline tubing, airstones, and airline gang valves
15. black plastic sheet
16. shade cloth
17. 10 µm filter bags

* = items that must be made

NOTE: THE QUANTITY OF ITEMS 2 to 6 ARE DEPENDENT UPON THE NUMBER OF REARING TANKS TO BE EMPLOYED.

The recommended larval rearing tank should be circular in shape, constructed out of fiberglass with a black epoxy coated interior, equipped with a center drain in which a two inch center standpipe can be fitted. It is preferred that the bottoms have a slight slope (i.e., 3° towards the center drain), however, this feature is not essential. The interior walls should be smooth. The center standpipe should be equipped with a coupling in which a short drain pipe with the sides cut out and screened with 250 µm can be inserted. As the larvae grow older and the rate of water exchange increases, this can be replaced with a similar cap with 500 µm screening.

The number or size of tanks can vary and is largely dependent on spacial restrictions. However, the number of rearing tanks employed in a rearing trial should be based upon the availability of oyster trochophores, phytoplankton and rotifer production outputs. Because of the large number of larvae resulting from a single spawn, there will be a desire to stock all of the available larvae.

Step 1. Tank Preparation: The larval rearing tanks and their components should be air dried when not in use. They must be assembled on the day of the second injection of the spawning sequence described previously. Rearing tanks should be

scrubbed and rinsed with fresh water (avoid using detergents or chlorine) and filled with filtered seawater (i.e., ≥ 32 ppt). The center drain should be capped with a 250 μm nytex cap and an airstone (preferably ring type) placed near the center drain. At least four additional airstones should be placed in separate quarters of the rearing tank to help keep the eggs suspended in the water column until they have hatched.

Step 2. Stage of Development for Stocking: When the embryos are in somite stage, (Final Report, Appendix 4, Plate N), they can be stocked into the larval-rearing tanks at an initial density of 30-40 larvae/l. After the eggs have been stocked, the rearing tanks should be covered with a black plastic sheet to prevent the hatched larvae from being exposed to sunlight. This should be maintained for at least three days posthatching at which time the larval tanks can be covered with a shade cloth.

▸ Extensive Larval Rearing

Preparation of the extensive rearing tank should commence approximately seven to eight days prior to receiving eggs and the operator must exercise proper planning in order to manage this type of rearing system effectively.

Materials Required:

1. 20,000-liter fiberglass rearing tank(s)
2. aeration and seawater system
3. shade cloth
4. black plastic sheet
5. waterproof tarp
6. 4" center stand pipe
7. 4" cap with 250 μm nytex screen
8. filter bag (10 μm)
9. 250 μm nytex bag

The tank should be located outdoors in a location where it can be easily stocked with phytoplankton. The inner sides of the tank should be black and smooth. Prior to a rearing trial, the tank should be scrubbed and rinsed with fresh water and allowed to air dry.

Step 1. Tank Preparation: Seven to eight days prior to receiving eggs, fill the tank with filtered seawater (32-35 ppt) to a depth of approximately one meter. Place an aeration ring or distribute airstones ($1/m^2$) and aerate the water in the tank. Above the tank (i.e., one meter) suspend a waterproof tarp to prevent rain water from entering during preparation of the tank and the larval rearing trial. Be sure that the tarp is properly secured.

Step 2. Inoculation of Phytoplankton: After filling the tank with seawater, inoculate it with phytoplankton to attain a density of 300 x 10^3 cells/ml. Be sure that the phytoplankton is also filtered using the filter bag. During the following days add phytoplankton as needed to maintain the initial density.

Step 3. Inoculation of Copepods: On the same day of inoculating phytoplankton, stock the tank with 80 x 10^3 copepods. Be sure that the copepods have been filtered through a 250 µm mesh net. Allow the copepods to multiply directly in the tank.

Step 4. Inoculation of Rotifers: Three to four days prior to stocking the eggs inoculate the tank with rotifers at a density of 5 rotifers/ml.

Step 5. Stocking of Eggs: When eggs become available they should be stocked into the rearing tank at a density of 10 eggs/l and allowed to hatch. The shade cloth should be stretched over the entire surface of the tank and firmly secured. The black plastic sheet should then be placed on top of the shade cloth and also secured. Allow three or four locations where the shade cloth can be easily lifted for daily monitoring activities.

Feeding Regimen

▸ Intensive Larval Rearing

A schematic representation of a generalized feeding regimen employed in the intensive rearing of grouper larvae (Tamaru et al., 1993a) is presented in Figure 5. It depicts the various feed organisms reported in the literature and when they should be introduced to the grouper larvae as feed.

Step 1. Inoculation of Phytoplankton: Phytoplankton is introduced into the larval-rearing tank on the second day posthatching to achieve an initial-cell density ranging between 300 and 500 x 10^3 cells/ml. Additional phytoplankton should be added to the rearing tank daily as needed to maintain the initial cell densities over the next 25 days of the larval rearing trial.

Step 2. Introduction of Oyster Trochophores: Beginning on the second day posthatching, oyster trochophores should be introduced into the larval-rearing tank to attain a density of 20 individuals/ml. This density should be maintained until day 10 posthatching. As seen in Figure 5, SS-type rotifers, mussel larvae, or sea urchin larvae can be used as substitutes if available.

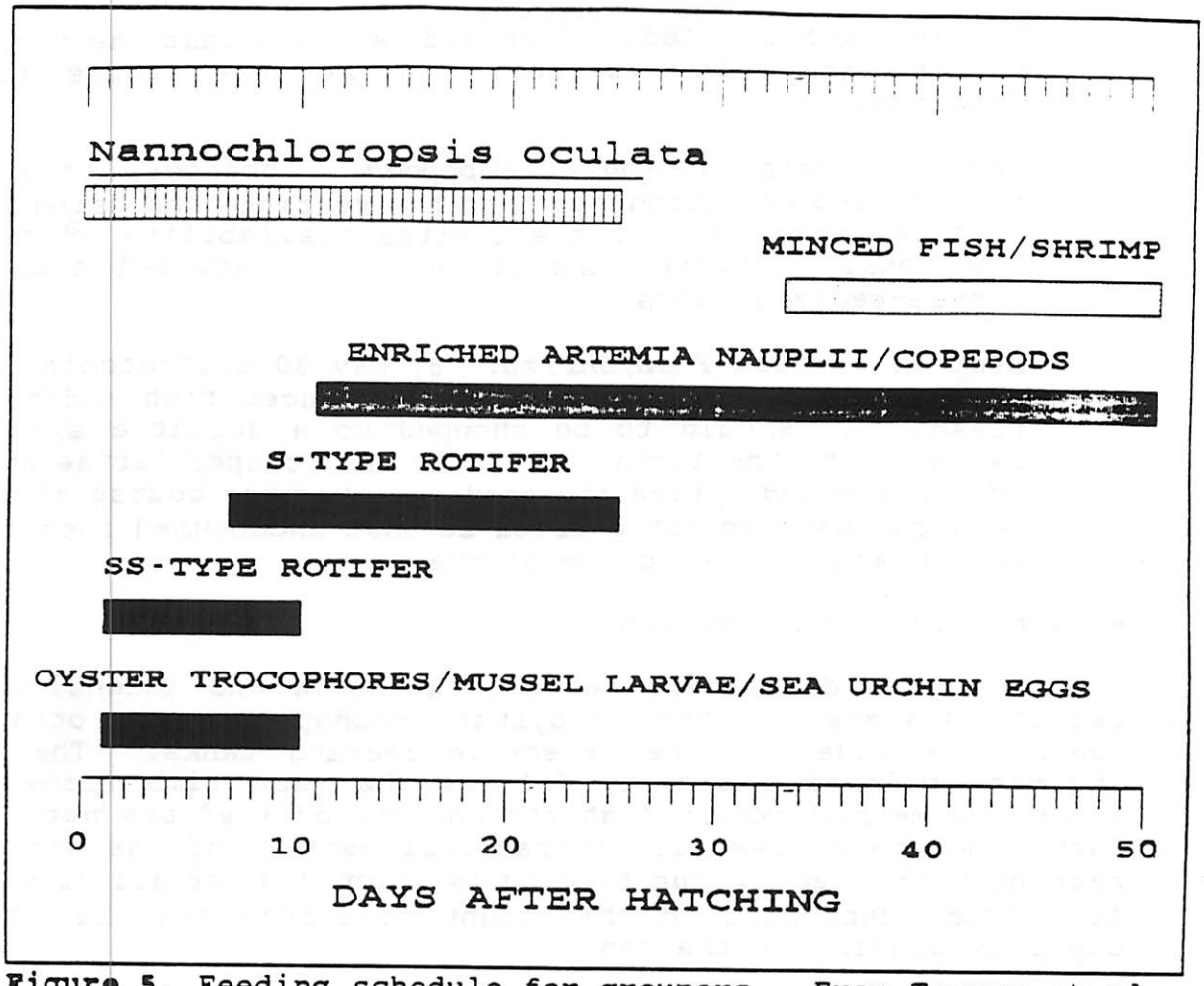

Figure 5. Feeding schedule for groupers. From Tamaru et al., 1993a.

Step 3. Introduction of Rotifers: On the second day posthatching, S-type rotifers can be introduced into the rearing tank. An initial density of 10-20 rotifers/ml is recommended. Rotifers are added as needed on a daily basis to maintain the initial stocking density.

Step 4. Introduction of Artemia Nauplii: Between Day 10 and 12 posthatching enriched *Artemia* nauplii can be provided to the larvae. An initial density of 0.01 nauplii/ml is recommended. At the end of each day, it should be determined whether all of the nauplii have been consumed. This will determine whether to increase the next day's ration. If all of the nauplii have been consumed, the number of nauplii provided the following day should be doubled. This process is repeated until a density of 2 or 3 nauplii/ml is attained. This density of nauplii should remain constant until the

larvae are harvested. It should be noted that the total daily ration should be separated into at least three different feedings.

Step 5. Introduction of Copepods: Copepods, if available should also be introduced into the rearing tank using the same protocol. Because of the limited availability of copepods, the total harvested should be evenly divided amongst the larval-rearing tanks.

Step 6. Minced Fish/Shrimp: By Day 30 posthatching, larvae should be large enough to consume minced fish and/or shrimp flesh. It should to be chopped to a suitable size before being fed to the larvae. Provide the grouper larvae this feed *ad libitum* and spread the feedings over the course of the day. Be sure they are not overfed so that unconsumed feed does not accumulate on the bottom of the tank.

▸ Extensive Larval Rearing

The main difference between extensive and intensive larval rearing systems is that no oyster trochophores (or other small feeds) are added to the extensive rearing tanks. The feeding regimen employed basically follows the same time frame as the intensive method except that the amounts of feed are not as high. Large amounts of feed are impractical because of the size of the rearing tank. *Ad libitum* feeding is practiced for all of the major feed items depending on the amount of larvae and the number of copepods growing in the tank.

Water Exchange

The same time frame for water exchange can be followed for both the intensive and extensive system. Again, the major difference will be the amount. Initially, no water exchange should occur during the first ten days posthatching. For the intensive system, a 20% rate of exchange can be initiated on Day 11 posthatching. The amount of exchange is gradually doubled with each successive day until a rate of 100%/day is attained. This rate is then maintained for the duration of the trial.

In the extensive system, it is again emphasized that the productivity of the pond provides the major source of the feed for the grouper larvae. An excessive rate of water exchange will result in flushing out the available feed. The operator will have to continually use his judgement on a daily basis as to whether to increase of decrease the rate of exchange. Beginning on Day 11 posthatching an initial 10% rate of exchange is recommended. Again the rate is doubled on a daily basis until a rate 75%/day is achieved. From this point on the 75% daily exchange rate should be maintained until the end of the trial.

Daily Routine

Daily monitoring of the larval-rearing systems is critical to the success of the rearing trial.

Materials Required:

1. DO meter
2. refractometer
3. thermometer
4. 1-ml rotifer counting pipette
5. 10x magnifier
6. data sheet
7. siphon hose (airline tubing and 1/2" i.d.)
8. 5' glass tubing (1/8" and 1/2" i.d.)

Step 1. Water Quality: Temperature, DO, and salinity should be measured daily. Record data so that it can be easily examined for trends.

Step 2. Rotifer Measurements: The rotifer densities in the tank should be measured in the morning and afternoon by taking five 1-ml aliquots at various locations in the tank. Calculate the average rotifer density. These values dictate whether addition of rotifers is warranted as well as whether the rate of water exchange is excessive. Adjustments can be made on a daily basis.

Step 3. Tank Maintenance: Although not the most pleasant activity during larval rearing but necessary none the less, is the siphoning of the tank bottom to remove dead larvae, uneaten food and accumulated debris. The tank bottom should **NEVER** be allowed to have an excessive amount of accumulated debris. This activity may prove to be more difficult with the outdoor tanks.

Trends in water quality measurements, feed levels, and conditions of the tank bottom must be summarized daily to assess the progress of the larvae. Based on the observations, adjustments can be made to minimize mortalities.

Sorting

A high degree of cannibalism during the rearing of grouper larvae has been reported to take place after Day 25 posthatching and mortalities can reach as high as 10%/day, especially with a rearing trial that results in a wide size distribution of larvae. This usually occurs in rearing trials with high stocking densities. For all practical purposes, sorting is restricted to intensive rearing systems because of the size of tanks as well as the densities encountered. Currently, the only means of reducing

losses as a result of cannibalism is to sort the la ae by size. This is an extremely labor intensive but necessary t. . which must be repeated at regular intervals (every two or three days) during the course of the rearing trial. The procedure for sorting is as follows:

Materials Required:
1. fine mesh barrier net
2. 20-liter plastic buckets
3. 5-l plastic buckets
4. aquarium dip nets

Step 1. Reduce the Volume of the Rearing Tank: All rearing tanks should have the facility to reduce its volume without loss of the larvae. This is accomplished by slowly opening the main drainage system of the rearing tank and/or using ring siphons.

Step 2. Concentrating the Larvae: Using the fine mesh barrier net, the larvae can be corralled into one area of the rearing tank. The larger individuals will usually be able to avoid the net and this is a means of separating the size classes.

Step 3. Removal of the Larvae: Determine which of the size groups are easiest to be removed from the rearing tank. Although fewer in number, the larger individuals are often more difficult to catch. In all cases, the larvae are susceptible to handling and precautions should be taken so that the larvae are not suspended out of water. The smaller individuals are removed by scooping with buckets and stocked into a separate rearing tank. Repeat until the sorting is completed. An alternative procedure is to purchase size sorters. The entire larval community can be placed directly within the sorters and the smaller individuals will swim between the bars of the sorters leaving behind the larger individuals. The larger individuals can then be removed and placed into a separate tank.

Stress Test

The stress test was developed to assess the health of hatchery produced larvae and juveniles and is currently an accepted quality control measure for both fish and crustaceans (Dhert et al., 1992). In the past, growth and survival were the only means of assessment. However, applying stress at various stages of larval development has become a major criteria used in evaluating the condition of the larvae during the rearing trials and/or determining when the larvae are able to withstand harvesting.

The stressors that are employed can be characterized into two categories: physical and physiological. Resistance to capture and

being suspended in air for a predetermined duration of time are examples of physical stressors and exposure to various salinities at predetermined time intervals is an example of a physiological stressor. It has been demonstrated that stress resistance can be used to detect differences in treatment effects where no differences can be found by survival or growth (Tamaru et al., 1993b). In addition, there appears to be a direct link between the stress resistance exhibited by larvae and the nutritional value of their feed (Tackaert et al., 1989, Dhert et al., 1992; Tamaru et al., 1993b).

The methodology for using physical stress as a means to determine the time of harvesting for striped mullet (Tamaru et al., 1993b) is described below and can be adapted for use with grouper.

Materials Required:

1. 20-liter buckets, two for each tank to be tested
2. air stones and airline tubing
3. aeration and seawater system
4. fine mesh aquarium dip nets
5. hand tally

Step 1. Application of Physical Stress: Fill the buckets with seawater and aerate the water in each. Catch approximately 25-50 individuals from the rearing tank with the scoop net and hold the larvae suspended in the air for 15 seconds. After the time has elapsed place the fish into one of the buckets. Repeat the exercise for the duplicate bucket. Keep the collected fish in the buckets for one hour.

Step 2. Recording Mortalities: After one hour has elapsed, count the dead fish and record the number. Count the number of fish still alive and return them to their respective rearing tanks.

Step 3. Estimation of Survival: Total the number of dead and surviving fish in each bucket. Divide the number of surviving fish by the total number of fish collected and multiply by 100 to give a percent survival. A summary of the calculation follows:

Bucket 1

(A) (# of fish dead)
\+ (# of fish alive)

Total # of fish

(B) (# of fish alive ÷ Total # of fish) x 100 = % survival for Bucket 1

Repeat for Bucket 2.

Average the percent survival from both buckets and record the data. When percent survival of the stress test is above 90%, the larvae are ready for harvest.

Harvesting

The major activity at the time of harvest is the quantification of the surviving larvae. If the numbers are not excessive this can be achieved by direct count. However, if the numbers of surviving larvae are high, estimations need to be used.

Materials Required:

1. 1 x 0.5 m fine mesh net stretched between two poles
2. hand tally
3. four 20-liter buckets marked with 1 liter increments
4. airstones and airline tubing
5. aquarium scoop nets
6. seawater and aeration systems
7. 100-ml plastic cups
8. four 2" diameter ring siphons (500 μm)

Step 1. Preparation for Harvest: Drain the rearing tank to 1/3 its volume as described previously and concentrate the fish using the large net.

Step 2. Preparation of Holding Buckets: Fill the 20-liter buckets with 10 liters of seawater and aerate.

Step 3. Preparation of Standard: Count out 1000 fry into one 20-liter bucket and mark it as the standard. Use the ring siphons to remove excess water and bring the volume back to 10 liters. *NOTE: THE DENSITY OF THE STANDARD BUCKET CAN BE CHANGED AT THE MANAGER'S DISCRETION*. Place an airstone and aerate.

Step 4. Quantifying the Fry: Place another 20-liter bucket next to the standard bucket and add fry until the density appears to be the same. Repeat this sequence and record the number of buckets removed from the tank. Multiply this number by 1000 to obtain the total number of fish harvested.

PARASITES AND DISEASE

Any organism in captivity undergoes a certain degree of stress due to the artificial conditions (e.g., food, space, water quality) under which it is forced to live. Accidental occurrences such as power outages or malfunctioning of equipment can cause acute stress (e.g., low DO levels, high ammonia). The organisms can die quickly and often in large numbers unless promptly relieved of the adverse conditions. A more subtle and often undetectable kind of stress is low level chronic stress that often results from neglect and

poor vigilance. This type of stress results in poor performance (i.e., growth, survival, reproduction) at all phases of the operations (Billard et al., 1981). It must be emphasized that poor performance alone is not the only outcome of chronic stress. Such conditions, if not remedied can lower the resistance of the organism and sets the stage for infection by pathogens.

First Aid

The administration of first aid is not directly related to disease, although it is an activity in which therapeutic treatments are conducted. These procedures are the same as those used in the treatment of infections and for that reason are introduced here. First aid is usually required for larger individuals such as broodstock and juveniles. Fish obtained from the wild or fish that have undergone physical handling (i.e., gonadal biopsy, induction of spawning) often suffer from acute stress and bodily injury (e.g., loss of scales, abrasions and cuts). If left unattended the cuts and abrasions can become infected and ultimately may lead to death. First aid treatment can reduce these unnecessary losses.

Materials Required:

1. top loading balance
2. 500 to 1000-liter tank
3. seawater, fresh water and aeration system
4. 5-liter bucket or pitcher
5. Prefuran (Argent Chemical Redmond Washington, USA)

Step 1. Treatment Tank: A separate tank with a continuous supply of seawater, fresh water, and aeration should be designated specifically for treating fish. The tank and the water systems should have the capacity to accommodate a rate of water exchange of 300 to 400%/day.

Step 2. Reagent: Antibacterial treatment is recommended if the wounds are large or appear to be infected. The recommended antibiotic is Prefuran which is a broad spectrum antibacterial effective against both gram positive/negative bacteria. It is often used as an antiseptic and/or disinfectant and is also reported to be active against coccidia type infections. It is usually administered as a bath (5 ppm) for one hour followed by flushing to remove the chemical. The treatment is repeated for five days. Follow the instructions that come with the reagent and calculate the amount of Prefuran required to result in a final concentration of the active ingredient of 5 ppm.

Step 3. Treatment: First turn off the water supply to the treatment tank. Dissolve the amount of Prefuran needed to treat the volume of water in the tank and distribute evenly

into the treatment tank. Keep the aeration on and leave the fish in the treatment for one hour. After the time has elapsed turn the water on to flush the tank. Repeat Steps 2 and 3 for five consecutive days. During this period of time the fish should be fed *ad libitum*, never allowing excess feed to accumulate in the tank.

***Caligus* sp. or Sea Lice**

Parasitic copepods of *Caligus* sp. were present on groupers captured from the wild. They are relatively large and can be seen with the naked eye when a broodstock individual is held in your hand. They are usually crawling over the entire body but are most easily seen on the head region. Low level infestations are quite common on wild fish and even on healthy broodstock. However, the populations of this parasite can explode into epidemic proportions within two to three weeks and must be treated before they reach this stage.

Materials Required:
1. top loading balance
2. 500 to 1000-liter tank
3. seawater, fresh water and aeration systems
4. 5-liter bucket or pitcher
5. Trichlorofon (Argent Chemical, Redmond Washington, USA)

Step 1. Isolation: If the number of infected broodstock is low they can be isolated into a treatment tank as described in the section on first aid. If there are a large number of infected individuals, the entire holding facility must be treated.

Step 2. Reagent: Trichlorfon (also known as dipteryx or masoten) has been found to be the most effective chemical treatment for dealing with *Caligus* sp. It is an organo-phosphate compound in whitish powder form (80-97% active ingredient). It is also effective on other metazoan parasites (e.g., leeches, monogenetic trematodes, and other parasitic protozoans). It is extremely toxic to invertebrates (especially shrimp, crabs and mollusks) and contact with the skin and inhalation should be avoided. This reagent breaks down rapidly when exposed to light, high temperature and high pH. For best results, the treatments should be initiated during the morning hours. *NOTE: IT IS UNLAWFUL TO TREAT FISH MEANT FOR HUMAN CONSUMPTION WITH THIS CHEMICAL.*

Step 3. Treatment: A one hour exposure to 0.5 ppm of trichlorfon is the recommended treatment. Determine the

amount of reagent needed using the equation below. The calculation for determining the amount of Trichlorfon (80% active ingredient) needed for a 1000-liter treatment tank is:

Trichlorfon = (1000 l) x (0.001 g/l) x 0.5 ppm x (100/80)

Based on the above calculation, 0.63 g of trichlorfon is required. Weigh out the amount and dissolve in five liters of water obtained from the treatment tank. Turn off the water supply and pour the reagent into the treatment tank, making sure that it is evenly distributed. Make sure that the aeration is on and treat the fish for one hour. After one hour, flush the tank. This treatment should be repeated as needed depending upon the quantity of parasites remaining. The holding facility from which the fish came should also be treated or cleaned and allowed to sun dry. When large number of broodstock are infected, the entire holding facility must be treated.

LITERATURE CITED

Bardach, J.E., J.H., Ryther and W.O. McLarney. 1972. Aquaculture: the farming and husbandry of freshwater and marine organisms. Wiley Interscience, New York, 868 pp.

Billard, R., C. Bry and C. Billet. 1981. Stress, environment, and reproduction in teleost fish. In: A.P. Pickering (ed.) Stress and Fish, pp. 185-208.

Dhert, P. P. Lavens, and P. Sorgeloos. 1992. Stress evaluation: a tool for quality control of hatchery-produced shrimp and fish fry. Aquaculture Europe, Vol. 17(2):6-10.

FitzGerald, William Jr., M. Bauerlein and T. Behrenfeld. 1991. Preliminary spawning and culture of groupers in Palau and Guam. Final Report, Pacific Aquaculture Association. Honolulu, Hawaii USA, pp. 1-53.

Fulks W. and K.L. Main. 1991. Rotifer and microalgae culture systems. Proceedings of a U.S.-Asia Workshop. Honolulu, Hawaii, January 28-31, 1991. Published by The Oceanic Institute, Honolulu, 364 pp.

Johannes, R.E. 1981. Words of the Lagoon: Fishing and Marine Lore in the Palau District of Micronesia. University of California Press, Berkely CA. 245 pp.

Lubzens, E. 1987. Raising rotifers for use in aquaculture. Hydrobiologia, 147:245-255.

Lubzens, E., A. Tandler and G. Minkoff. 1989. Rotifers as food in aquaculture. Hydrobiologia. 186/187: 387-400.

Myers, R.F. 1991. Micronesian Reef Fishes. A practical guide to the identification of the coral reef fishes of the tropical central and Western Pacific. Coral Graphics, Barrigada, Territory of Guam. 298 pp.

Sorgeloos, P., P. Lavens, Ph. Leger, W. Tackert and D. Versichele. 1986. Manual for the culture and use of brine shrimp *Artemia* in aquaculture. *Artemia* Reference Center, State University of Ghent, Belgium. 319 pp.

Sorgeloos, P. P. Lavens, Ph. Leger and W. Tackaert. 1991. The use of *Artemia* in marine fish culture. Paper presented at : Finfish in Asia '91 Conference, Tungkang, Taiwan. Dec. 17-19, 1991. 12 pp.

Sweetman, J.W. 1993. Perspectives and critical success factors in the present farming of fish. Paper presented at World Aquaculture '93, Torremolinos, Spain, May 26-28, 1993. European Aquaculture Society, Special Publication No. 19. Oostende, Belgium. pp. 288.

Tackaert, W., P. Abelin, Ph. Leger and P. Sorgeloos. 1989. Stress resistance as a criterium to evaluate quality of postlarval shrimp reared under different feeding procedures: In: Proceedings III Simposio Brasileiro sobre cultivo de camarao. VOl. 1. MCR Aquacultura, Joao Pesoa, Brasil. pp. 393-403.

Tamaru, C.S., C.-S. Lee and H. Ako. 1991. Improving the larval rearing of striped mullet, (Mugil cephalus) by manipulating quantity and quality of the rotifer, Brachionus plicatilus. In: Rotifer and microalgae culture systems. Proceedings of a U.S. - Asia workshop. (Fulks, W. and Main, K. Eds.). The Oceanic Institute, Hawaii, USA, pp.89-103.

Tamaru, C.S., F. Cholik, J.C.-M. Kuo and W. FitzGerald, Jr. 1993a. Status of the culture for striped mullet (*Mugil cephalus*) milkfish (*Chanos chanos*) and Grouper (*Epinephelus* sp.). World Aquaculture '93, Torremolinos, Spain, May 26-28. European Aquaculture Society, Special Publication No. 19. pp. 296-297.

Tamaru, C.S., W. J. FitzGerald, Jr. and V.S. Sato. 1993b. In: Christine Carlstrom-Trick (Ed.), Manual for the artificial propagation of striped mullet (*Mugil cephalus*). Guam Aquaculture Development and Training Center. 165 pp. (In press).

Watanabe, T., C. Kitajima and S. Fujita. 1983. Nutritional values of live food organisms used in Japan for mass propagation of fish: A Review. Aquaculture, 34:115-143.