Czapex-Dox and pH3 to 10 in SD agars, in both cases viable temperature ranged from 20 to 40 °C.

Table 3.- Comparation of pH and temperature characteristics of HBMC -314 strain

| pH for growth | Czapex-Dox agar | Sabouraud-dextrose agar |
|---|---|---|
| 2 | - | + |
| 4 | + | + |
| 6 | + | + |
| 8 | + | + |
| 0 | - | + |
| 12 | - | + |
| Temperature (°C) for growth | | |
| 15 | - | - |
| 20 | + | + |
| 25 | + | + |
| 30 | + | + |
| 35 | + | + |
| 45 | + | + |

(+) = grown
(-) = no grown

## Discussion

The main characteristic of *Aspergillus sp.* is the production of red pigment on the substrate (red colour observed on commercial diet ) this was seen in the strain HBMC-314. These colonies were grown in pH 2 to 10, and at temperature range from 15 to 45°C; *The Aspergillus sp* are mesophilic, Banwart. (1979).

The strain HBMC-314 taken from a pond culture which was fed with same trial diet, was identified according to its reproductive morphology as *Aspergillus flavus* . The floccose shape is a typical characteristic of this strain, in which the conidiophores leave directly from the substrate and develop in close proximity to each other.. The colonies on Czapek-Dox were olive green and in front of light , were dark green, and the sterigmata were both

uniseriated and biseriated. Asexual reproduction was observed typical of Deuteromicotina ( fungi imperfect).

*A. parasiticus* also a producer of aflatoxin B1, presented the same characteristic morphology as that of *A. flavus*, but the colour of the colonies was a different intensity of green, and the sterigmata were single and seriade on Czapex-Dox agar.

Another fungus similar to *A. flavus* is *A. oryzae*, but it does not produce aflatoxin, and its culture colonies do not normally show a green colour.

A definitive characteristic for the identification of *A. flavus* from other morphologically similar Aspergillus sp such as *A. parasiticus* and *A. oryzae* is the green colour of the conidia, and change of colour from green to yellow in amonium atmosphere, and the retourn to green colour in acetic acid atmosphere. This characteristic is not demostrated with other types of *Aspergillus*.

The reproductive morphology of the dark brown HBMC - 322 strain identifies it as *A. niger*; Dark brown conidia and other characteristics such as the low number of: sterigmata on the chain conidies , lighter red conidiophoros, the high head shaped vesicle and the lighter coloured sclerothia.,help to indentify this species. *Penicillium sp* (fig. 8) was observed growing in the isolates of *A.niger* , and is considered as parasite of this particular *Aspergillus* (Thom and Raper 1945), and therefore also help in identication. The *A. niger* has not been demonstrated to be a producer of aflatoxin B 1, Webster (1977).

The *A. flavus* colonies presented different colours occording to the media type, but they did not vary in their basic morphology This indicated that this strain can mutate so altering the biochemistry of the particular serotype present. The *A. niger* colonies do not vary their biochemistry on different media of culture, which may indicate that this strain is less susceptible to mutation.

Biochemical characterization of *A. flavus* with carbohydrate showed that it was not able to produce fermentation on lactose, manitol, mioinositol, D-sorbitol substrates but was able to asimilate these with the single exception of arabinose .Urea was also shown to be an energy source. The *A. flavus* not able to descompose alginic and tartaric acids.The present study has allowed to established a caracteristic biochemical pattern of the carbohydrate metabolism of the HBMC - 314 strain identified as *A. flavus*.. No previous studies or literature have been discribing the biochemical caracteristics for *Aspergillus sp.* serotypes.

The biochemical characteristics of *A. niger* showed no arabinose, D-sorbitol, manitol and urea asimilation. (laskin and Lechavalier 1978).The *A.*

*niger* not able to descompose and to assimilate tartaric and alginic acids, sodium citrate.

In conclusion the hepatopancreas superficial membrane necrosis was possibly caused by the aflatoxin of *A. flavus* fungi, based on the presence of the pathogenic agent an the artificial diet and it is characteristic expression in the test shrimp.

Acknowledgment.. To Tecn. Alim. Paula Pinto Bonilla for her aid reviewing this work.To Stanislaus Sonnenholzner for supervision of work.To Phillip Buicke for the translation to English.

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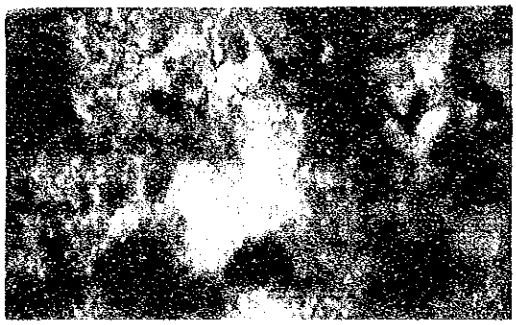

Fig. 1 Artificial Food with molds

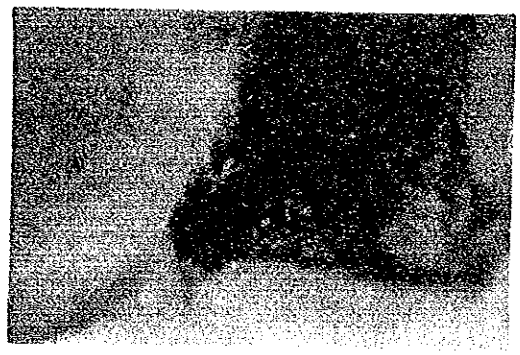

Fig. 2 Surface necrosis of hepatopnacreas

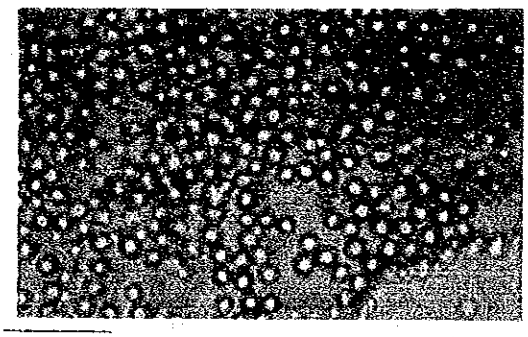

Fig. 3 Conidie spores of A. flavus

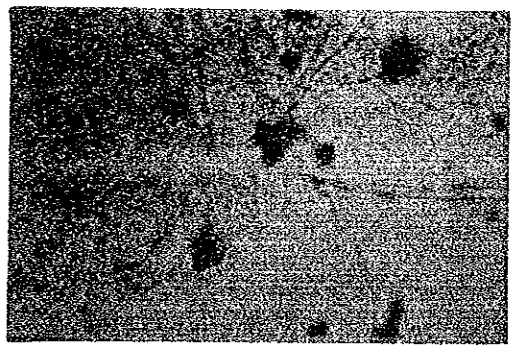

Fig. 4 A. flavus on Saboroud dextrose

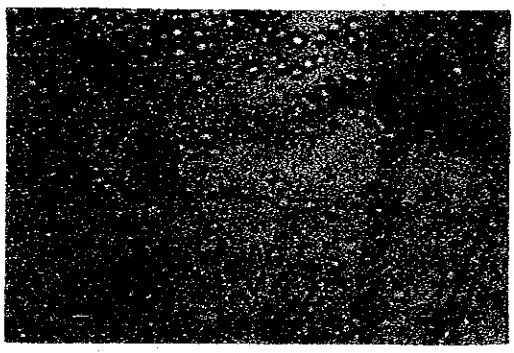

Fig. 5 A flavus on Czapex-Dox

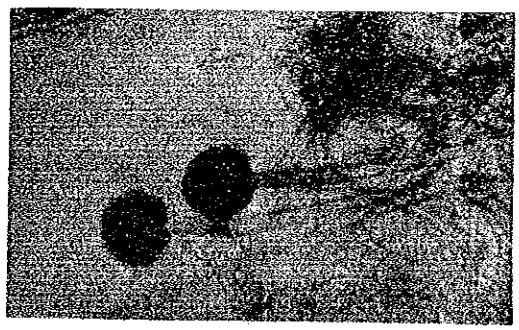

Fig. 6 A. niger on Sabouraud dextrose

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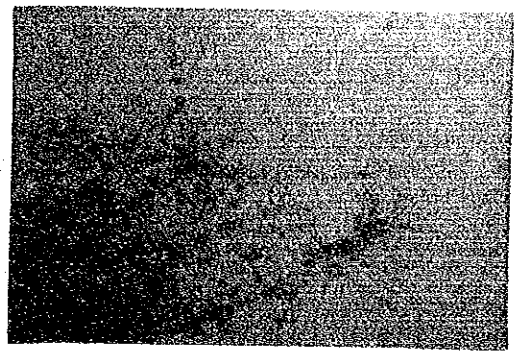

Fig. 7 Hyphae development of A. niger

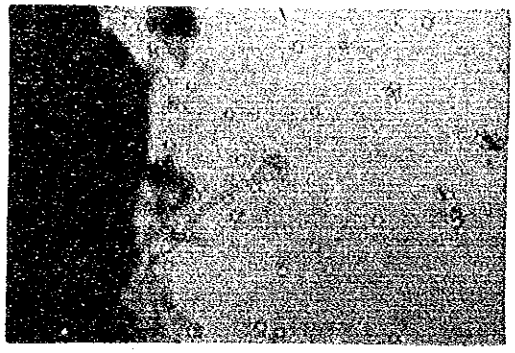

Fig. 8 Penicillium sp. on Sabouraud dextrose

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# ACTIVITIES REPORT "AQUATIC NUTRITION"

INTRODUCTION

The aquatic nutrition project included three stages within the master program:

— Basic Methodology
— Nutritional Requirements
— Feeding experiments

## 1.0 BASIC METHODOLOGY

— Practical acquirement in use and maintenance of equipment.
— Practical acquirement of biochemical analysis.

The main goal is to establish techniques and analytical methods to characterize lipids, carbohydrates, proteins, vitamins and some other components that encompass the nutritional requirements of shrimps.

Several activities were involved:

Calibration, Maintenance and use of :
a) Gas Chromatograph (GC)
b) Liquid Chromatograph (HPLC)
c) Spectrophotometer
d) Spectrofluormeter
e) Iatroscan

The equipment listed above are high sophisticated instruments which require a theoretical/practical training for the different analysis. It is important to know not only the use, but also the calibration of the equipment for the different methodologies.

### 1.1 Practical acquirement in use and maintenance of equipments

#### 1.1.1 GC Calibration

Several tests for the Shimadzu GC 14A were done for the neumatic system (air, nitrogen, hydrogen) as well as the control valves and to obtain optimal pressures. Its columns were filled, installed and activated. The following tests were conducted:

- Determination of the optimal flow for the gas carrier (HETP)
- Determination of the hydrogen optimal flow
- Determination of the air optimal flow
- Baseline stabilization
- Column Charge Capacity
- Calibration with standards PUFA-1, PUFA-2, GLC-10

*Tests of Fatty acid standards*

Test of fatty acids standards (PUFA-1, PUFA-2, GLC-10, RM-3) were done with concentrations of 20-25 µg/µl, under laboratory conditions to establish the column and instrumental analysis that allow the identification and quantification of the components injected in the GC.

For the qualitative analysis, the concentrations given by the chromatograma were compared with those given by the standard. From the analysis we could deduce if there was a good qualitative response of the detector, and if there was a logic sequence of the distribution of correction factors obtained for the flame ionization detector (FID).

*Linearity of the Flame Ionization Detector (FID)*

To determine the response of the FID, a known standard were injected with concentrations of 2.2 and 11 µg/µl, obtaining a linear relationship ($r^2$=1) between the detector's answer and the standard, given by the sum of areas. As a result we can conclude that the FID detector has a good reponse for concentrations between 2.2 and 11 µg/µl.

*Height equivalent of theoretical plates (HETP)*

Once the HETP's values for different applied hydrogen and air flows were established, the optimum flow rate for a 2 m column was 54 ml/min. The best flow rates for the flame ionization detector (FID) were:

| | |
|---|---|
| Hydrogen | 63.63 ml/min |
| Air | 500.00 ml/min |

*Linearity and capacity of the Detector*

Fatty acid standard solvents were injected in order to get the column's capacity and detector linearity. The acceptable range lies between 1, 2 and 3 µl.

1.1.2 HPLC Calibration

Dr. Hiroshi Ogata visited CENAIM during April 1991. He gave advise about kinetics/spectral response activities, system analysis operation as well as maintenance of HPLC equipment. Due to the lack of some specific chemicals and standards, it was not possible to carry out the practical training and calibration of the HPLC.

The calibration of the equipment was done by Eng. Katsushi Tabata, expert on instrumental analysis and technician from Shimadzu Company. He gave a two days training to calibrate the Shimadzu HPLC 6A Liquid Chromatography. Once the equipment was calibrated with standard samples, an analysis of amino acids from a papaya sample was carried out to verify the good working of the equipment.

A technical bulletin in reference to the amino acid analysis with HPLC was prepared by the nutrition personel. It contains all the basic steps to carry out the analysis, begining with principles of amino acid analysis, preparation of chemicals and samples, standard preparation and other important methodologies.

1.1.3 Spectrophotometer Calibration

Two methods for UV/VIS Spectrophotometer calibration were implemented, prior to the analysis of phosphorus, chromium oxide, inorganic nutrients, proteins and enzymes.

1. Calibration by means of Dydimium Filter.
2. Calibration through standard curves fo specific components to be analyzed.

The calibration is repeated every 3 months for every component to be analyzed. This equipment is currently used for all the above mentioned analysis.

**1.2 Biochemical analysis**

The nutritional elements and organic substances needed for feeding bioaquatic species are to be known. Therefore, during the last two years, several techniques have been implemented at the nutrition lab, and they are the following:

- Lipid extraction (FAMES)
- Use of Buchi equipment for protein determination
- Ethereal Extraction
- Crude Protein
- Total phosphorous
- Ashes
- Crude Fiber

- Oil: peroxides and acidity
- Insolubles ashes
- Chrome oxide
- Mixed indexed for Sodium Chloride

1.2.1 Lipid Extraction (FAMES)

The chromatography is a high resolution method that requires complementary extraction techniques. Several extraction and lipid (FAMEs) isolation techniques have been established. The techniques already evaluated and regulary used at the nutrition laboratory are:

1. Folch (modified)
2. Bligh & Dyer (Kanazawa)
3. Transesterification (Ackman)
4. Acid Metilation

*Folch Modified Method*

Tests of lipid isolation were carried out by means of this method. Replicate samples (21) of a commercial diet (Higashimaru) gave a lipid mean value of 16.6% ±.69 S.D %.

*Bligh & Dyer Method*

The lipid isolation technique refered as Bligh & Dyer method and modified by Kanazawa (Kagoshima University) was also tested in the nutrition laboratory. For this purpose 10 replicates samples of a commercial diet were analyzed with this method and Folch modified method. The results are shown in the following table.

TABLE 1. COMPARISON OF TWO LIPID ISOLATION TECHNIQUES

| Method | Mean value (%) | Std. deviation (%) |
|---|---|---|
| Bligh & Dyer | 9.0 | ±0.43 |
| Folch Modified | 7.6 | ±0.60 |

The values obtained by the Bligh & Dyer method are relatively higher than the ones obtained by the Folch Modified method. These differences are due to the lack of cleaning procedures (Folch modified method does not made an extract cleaning). Besides, this method requires relatively expensive reagents.

*Transestherificación method*

Transestherification method (Ackman, 1987) and the acid methilation method were implemented in the nutrition laboratory, in order to obtain fatty acid methyl esthers (FAMEs), which were analyzed with gas chromatography. Chromatograms from both methods for the same sample showed little variation in the fatty acid composition (± 0.002%). Due to this fact, it is proved that the acid methilation technique will not change the chain compositon, either short or polyunsaturated. Additionally, this method do not use saponification reagents like OHNa and BF3 in methanol. We conclude that both methods, either acid methilation or transestherification can be used to obtain lipid profiles.

Several tests have been done to preserve the samples and to standardize materials (pear glasses) utilized during lipid extraction. In order to study the preservation of samples, fresh and frozen (-30°C) hepatopancreas were compared by means of their lipid profile. The results show a 0.03% difference of total lipids among fresh and stored hepatopancreas. The appropriate use of preservation methods (temperature, protection from sunlight and environment, anti-oxidants) allow to obtain similar results between frozen and fresh animal tissue.

1.2.2 Crude Protein

Several modifications of the Kjehldal technique were adapted for the Protein determinator Buchi. The assays consisted basically on testing several catalyzers, acids and bases which that take part in the reactions, as well as different sample sizes. One of the limitations of this method is its inability to detect protein levels smaller than 10mg, which correspond to nauplii, larvae or artemia. Actually, two techniques are used in the nutrition laboratory to determine crude protein: 1) INEN 543 (Instituto Nacional de Normalización-Ecuador) for foodstuff and animal tissue, and 2) Lowry's technique for protein determination in animal tissue, within a range of 30 to 100 mg, with a ±10 % precision.

1.2.3 Others

Table 2 shows the techniques implemented.

TABLE 2. NUTRITION ANALYSIS TECHNIQUES

| Method | Reference |
|---|---|
| Moisture | INEN 540 |
| Ether Extract | INEN 541 |
| Ash | INEN 544 |
| Crude Protein | INEN 543 |
| Crude Protein (small samples) | Lowry |
| Total Phosphorus | AOAC method |
| Crude Fiber | Modified Weende method |
| Acidity in oils | INEN 38-72 |
| Peroxide in oils | International Organization for Standarization 3960-1977 |
| Insoluble Ash | INEN 469 |
| Calcium (Permanganometric method) | INEN 546 |
| Sodium Chlorine | INEN |
| Chromium Oxide | Furukawa and Tsukara method |

Note: INEN = Instituto Nacional de Normalización
AOAC = Association of Analytical Chemistry

All the processes required for the establishment of the methodologies previously mentioned are fully described (step by step) in the quarterly reports to the CENAIM coordination.

## 2.0 NUTRITIONAL REQUIREMENTS

The second phase of the nutrition program comprised the establishment of nutritional requirements for *P. vannamei* in order to reach good growth and health during its different stages. This was achieved through the study of protein and lipid levels within feeding experiments. Some of the diets were formulated and prepared at CENAIM.

The first step was to review the available literature related to nutritional requirements of proteins, lipids and carbohidrates in penaied shrimps. The nutritional requirements for larvae and adults of

*P. vannamei* were determined during the research project entitled "Investigaciones Bioquímicas Nutricionales (IBN)" conducted at ESPOL by the School of Marine Engineering and Sea Science (ESPOL). These are listed below.

TABLE 3. PENAEID SHRIMP NUTRITIONAL REQUIREMENTS

| Parameters | Maturation % | Larviculture % |
|---|---|---|
| Protein | 45-50 | 50-55 |
| Lipids | 10-12 | 10-15 |
| Fiber | 2-3 | 0-1 |
| Ash | 10-15 | 5-? |
| Moisture | 10-? | 5-8 |
| Calcium/Phosphorus | 2:1 | 2:1 |
| Vegetable/Animal | 1:5 | 1:5 |

Two experiments have been carried out with *P. vannamei*, in order to determine:

i) Quality of diets prepared with local materials, as food for shrimp broodstock and larvae of *P. vannamei*.
ii) Protein level requirement of *P. vannamei* juveniles.

*Experiment 1*

One diet for *P. vannamei* broodstock and another for *P. vannamei* larvae were elaborated. The first diet was compared with fresh food (oysters) and a commercial diet (Nippai), while the second one could not be tested due to bacterial infection on the larvae culture tanks.

The diets were elaborated based on bromatological and biochemical information on lipids (FAMES) of selected raw materials. It was taken into account the information about nutritional requirements of penaeids shrimp. This first assay allowed the personnel from the nutrition division to become familiar with the management and use of the feed meal equipment. At the same time they acquire experience in the elaboration of diets of different size and formulation. Finally, through the same experiment they obtained experience in shrimp larval rearing.

The experimental design does not allow an statistical analysis, therefore, we could not establish significant differences between the diets in terms of production, nauplii and larvae quality, percentage of hatching, etc.

The maturation diet has an 8 hour stability in seawater. Its size was 10mm length by 2.5 mm diameter. Fresh food ration was 10% of the biomass. Feeding rate was 17% of the biomass, distributed according with the next table:

TABLE 4. EXPERIMENTAL RATION COMPOSITION (%)

| | DIET 1 | DIET 2 | DIET 3 | CONTROL |
|---|---|---|---|---|
| M001 | 1.8 | -- | -- | -- |
| M002 | -- | 1.8 | -- | -- |
| Nippai | -- | -- | 1.8 | -- |
| Oyster | -- | -- | -- | 1.8 |
| Calamar | 4.0 | 4.0 | 4.0 | 4.0 |
| Mejillon | 3.0 | 3.0 | 3.0 | 3.0 |
| Almeja | 5.0 | 5.0 | 5.0 | 5.0 |
| Krill | 3.2 | 3.2 | 3.2 | 3.2 |

The nutritional parameters differ at the beginning and at the end of the experiment (lipids, carbohydrates and proteins). This is suspected to be due to storage conditions. For example, the table shows a decrease in the carbohydrate levels at the end of the experiment for the maturation diet M002. The following tables show the most relevant results, which are fully described in the internal reports at CENAIM.

TABLE 5: LOCAL FEEDSTUFF COMPOSITION

| Foodstuff | Moist % | Protein % | Lipids % | Fiber % | Ash % | Insoluble Ash % | Calcium % | Phosph % | CHO % |
|---|---|---|---|---|---|---|---|---|---|
| Fish meal | 8.32 | 68.86 | 11.10 | 0.15 | 19.98 | 0.04 | 7.24 | 3.74 | |
| Wheat bran | 10.45 | 14.47 | 6.54 | 16.63 | 6.42 | | 0.15 | 1.00 | 55.94 |
| Rice bran | 9.60 | 16.28 | 17.25 | 8.05 | 8.40 | | 0.32 | 1.54 | 50.02 |
| Soy lecithin | 0.05 | | 73.10 | | | | 7.27 | | |
| Shrimp meal | 16.49 | 50.12 | 9.67 | 10.02 | 27.91 | | | 2.33 | 21.28 |
| Soybean oil | 0.11 | | 97.7 | | | | | | |
| Soybean paste | 10.49 | 44.46 | 3.64 | 9.33 | 9.26 | | 1.50 | 0.74 | 33.31 |
| Corn oil | 11.21 | 10.50 | 5.15 | 2.44 | 1.60 | | traces | 0.32 | 80.31 |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Fish oil | 0.03 | | 97.31 | | | | | | |
| Squid meal | 11.07 | 86.62 | 8.53 | | 8.94 | | 0.18 | 1.04 | 1.91 |
| Krill meal | 16.85 | 68.49 | 15.40 | | 15.87 | | 1.91 | 1.79 | 0.24 |
| Dietetic lecithin | 6.53 | | 21.41 | | | | | | |

TABLE 6. CENAIM & COMMERCIAL DIETS COMPOSITION (AT THE BEGINING AND END OF EXPERIMENT)

| Diets | Frippak | CENAIM L001 | | CENAIM M001 | | CENAIM M002 | | Nippai | |
|---|---|---|---|---|---|---|---|---|---|
| | % | % | | % | | % | | % | |
| Parameters | | Begin | End | Begin | End | Begin | End | Begin | End |
| Moisture | 7.29 | 11.90 | 6.24 | 10.04 | 8.56 | 4.05 | 9.72 | 11.42 | 8.92 |
| Protein | 52.69 | 51.79 | 49.45 | 60.68 | 60.35 | 52.81 | 54.70 | 56.48 | 48.31 |
| Lipids | 17.96 | 13.87 | 13.83 | 10.66 | 10.44 | 17.29 | 14.15 | 13.74 | 10.35 |
| Fiber | | 1.02 | 0.83 | 3.25 | 3.76 | 3.68 | 3.98 | 4.30 | 1.70 |
| Ashes | 13.99 | 21.06 | 20.85 | 18.22 | 17.88 | 17.14 | 18.19 | 18.52 | 17.65 |
| Calcium | 3.27 | 3.78 | 4.07 | 3.09 | 2.96 | 4.35 | 4.30 | 4.19 | 4.00 |
| Phosphorus | 1.86 | 2.97 | 3.06 | 2.87 | 2.66 | 2.35 | 2.33 | 2.61 | 2.61 |
| Carbohidrates | | 12.26 | 15.04 | 7.19 | 7.57 | 9.08 | 8.98 | 6.96 | 21.99 |
| Insol. Ashes | 0.15 | 2.17 | 1.94 | 0.65 | 0.62 | 1.98 | 1.89 | 2.09 | 0.79 |

Note: Diet L001 for larviculture
Diets M001 & M002 for maturation

TABLE 7. LIPID AND FATTY ACID (N3 & N4) CONTENT OF *P. VANNAMEI* BROODSTOCK HEPATOPANCREAS.

| Diets | M001 (n=3) | | M002 (n=3) | | Nippai (n=20) | | Fresh (n=2) | |
|---|---|---|---|---|---|---|---|---|
| | % | | % | | % | | % | |
| Stage | III | IV | III | IV | III | IV | III | IV |
| Lipids | 9.36 | 15.42 | 21.32 | 16.43 | 14.04 | 13.47 | 13.86 | 28.75 |
| $\Sigma$n3 | 20.87 | 21.42 | 23.19 | 22.95 | 23.17 | 24.58 | 27.42 | 27.14 |
| $\Sigma$n6 | 5.32 | 6.63 | 5.38 | 5.21 | 6.28 | 5.73 | 6.26 | 4.98 |

TABLE 8. LIPID AND FATTY ACID COMPOSITION OF *P. VANNAMEI* NAUPLII

| Diets | M001 (n=5) | M002 (n=6) | Nippai (n=2) | Fresh (n=4) |
|---|---|---|---|---|
| | % | % | % | % |

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| | | | | |
|---|---|---|---|---|
| Moisture | 86.69 ± 2.10 | 86.96 ± 2.86 | 88.34 ± 0.19 | 88.32 ± 0.34 |
| Lipids | 22.30 ± 2.78 | 23.38 ± 3.70 | 26.61 ± 0.08 | 22.85 ± 1.87 |
| 20:5n-3 | 9.19 ± 1.29 | 9.05 ± 0.84 | 9.78 ± 1.07 | 9.81 ± 0.38 |
| 22:6n-3 | 12.01 ± 1.04 | 11.86 ± 0.84 | 11.84 ± 0.62 | 11.96 ± 1.23 |

*Experiment 2*

It was designed to determine the protein requirements for optimum growth and survival of *P. vannamei* juveniles.

*P. vannamei* juveniles with an average weight of 0.93 g were fed artificial diets, each with a different protein level. Protein content varied between 25% and 45 % at 5% intervals. After 6 weeks the best growth were obtained with the 40% protein diet.

The animals fed with the lowest protein content in the diets (25, 30 and 35%) showed significantly smaller (95%) body weight gains, 4.11, 3.50 and 3.57 g respectively. However, lower survival rates were found with the highest protein levels (68 y 77%). Some of these results are shown in Table 9.

TABLE 9. AVERAGE WEIGHT, SURVIVAL, AND FEED CONVERSION FOR *P. VANNAMEI* JUVENILES.

| DETERMINACIONES | DIETAS EXPERIMENTALES | | | | |
|---|---|---|---|---|---|
| | 25% Protein | 30% Protein | 35% Protein | 40% Protein | 45% Protein |
| Initial body weight (g) | 0.93 ±0.01a | 0.91 ±0.01a | 0.94 ±0.02a | 0.94 ±0.02a | 0.94 ±0.02a |
| Final body weight (g) | 4.11 ±0.15a | 3.50 ±0.11a | 3.57 ±0.15a | 6.57 ±0.24b | 6.52 ±0.22b |
| Weight gain/week (g) | 0.53 a | 0.43 a | 0.44 a | 0.94 b | 0.93 b |
| Average growth (mg/dia) | 78.00 a | 63.00 a | 64.00 a | 137.00 b | 136 b |
| Survival percentage | 83.00 | 82.00 | 94.00 | 68.00 | 77.00 |
| Total food supplied (g) | 814.00 | 860.00 | 955.00 | 1099.00 | 1036.00 |
| Total protein supplied (g) | 230.00 | 280.00 | 344.00 | 442.00 | 477.00 |
| Biomass (g) | 308.00 | 259.00 | 303.00 | 401.00 | 450.00 |
| Feed conversion factor (FCR) | 2.64 a | 3.32 b | 3.15 b | 2.77 a | 2.30 a |
| Protein efficiency (PER) | 1.34 b | 0.93 a | 0.88 a | 0.90 a | 0.94 a |

The treatments that includes the same letter have not shown significant differences ($P<0.05$). The conclusions and discussion will be presented in an accompanying scientific paper.

*Experiment 3*

This experiment was designed to determine the growth rate of *P. vannamei* juveniles, fed with commercial diets available in the ecuadorian market and the CENAIM diet containing 40% protein.

TABLE 10. DIETS PROTEIN CONTENT

| Diets | Protein content % |
|---|---|
| CENAIM | 40 |
| E | 38 |
| A | 38 |
| B | 38 |
| D | 35 |

After two weeks, animals fed with diets B and CENAIM showed higher average weights (Table 11) than the other three diets. The same behavior continued until the fourth week. Survival percentage was smaller for diets A and D.

TABLE 11. AVERAGE WEIGHT AND SURVIVAL PERCENTAGE FOR *P. VANNAMEI* JUVENILES (N=42)

| Diets | Weight (g) | | | Survival |
|---|---|---|---|---|
| | Initial | 2 weeks | 4 weeks | % |
| CENAIM | 1.53 ± 0.13 | 2.95 ± 0.41 | 4.67 ± 0.59 | 92.6 |
| E | 1.51 ± 0.13 | 2.30 ± 0.43 | 3.14 ± 0.48 | 93.8 |
| A | 1.49 ± 0.13 | 2.53 ± 0.61 | 3.58 ± 0.81 | 80.3 |
| B | 1.53 ± 0.14 | 3.34 ± 0.79 | 5.22 ± 0.96 | 93.8 |
| D | 1.50 ± 0.26 | 2.49 ± 0.64 | 3.52 ± 0.76 | 76.5 |

Feed conversion factor, growth rate, and daily weight gain are shown in Table 12. The best conversion factors correspond to animals fed with CENAIM and B diets. A similar behavior was observed in the growth rate.

TABLE 12. FEED CONVERSION FACTOR, GROWTH RATE AND DAILY WEIGHT FOR *P. VANNAMEI* JUVENILES (N=42).

| Diets | Food Conversion Factor | | Growth rate | | Weight (mg/day) | |
|---|---|---|---|---|---|---|
| | 2nd Week | 4th Week | 2nd Week | 4th Week | 2nd Week | 4thWeek |
| CENAIM | 1.16 | 1.64 | 1.42 | 1.72 | 101.43 | 112.14 |
| E | 1.45 | 2.11 | 0.79 | 0.84 | 56.43 | 58.21 |
| A | 1.43 | 2.15 | 1.04 | 1.05 | 74.29 | 74.64 |
| B | 0.99 | 1.57 | 1.81 | 1.88 | 129.28 | 132.86 |
| D | 1.42 | 2.32 | 0.99 | 1.03 | 70.71 | 72.14 |

## 3.0 FEEDING

The third stage of the program was "Feeding", characterized by the elaboration of diets with local raw materials. Several activities were performed and they are listed below:

1) determination of operational capacity of the feed meal equipments,
2) fish meal elaboration (squid and krill),
3) formulation and preparation of diets,
4) bromatological and biochemical control of row materials and fish meal.

*Installment and use of equipments in pilot plant*

The following equipments were installed in the pilot plant in order to proceed with the elaboration of artificial feed.

| Equipment | Model | Company |
|---|---|---|
| Impact Pulverizer | CVJ-PF1 | Nakayasu Co |
| Pulverizer | IP-1 | Nakayasu Co |
| KING Pellet dryer | KF | Nakayasu Co |
| Chopper(picadora) | King System | Nakayasu Co |
| Pelletizer | KO-42G | Nakayasu Co |
| Mixer | H-85 | Kokusan Co. |
| Siever | RB-8 | Nakayasu Co |
| Granuladora | 230-7.5GP | Nakayasu Co |

### 3.1 Determination of the operational capacity of equipments

The equipments operational capacity were determined after several tests with raw materials and fish meal

| Equipment | Capacity kg/hr |
|---|---|
| Impact Pulverizer | 1.00 |
| Pulverizer | 0.25 |
| KING Pellet dryer | 24.00 |
| Chopper | 2.00 |

### 3.2 Elaboration of squid and krill meal

Some of the basic elements to be included during the elaboration of artificial diets are composed by squid, krill and shrimp heads. Nevertheless, there are difficulties in obtaining these ingredients at an appropriate particle size, therefore, it was decided to obtain their flours by mean a liophilization process.

*Flour liophilization*

Prior to the liophilization process, squid (*Loligo sp.*), krill and shrimp heads must be homogenized and froze at -20°C. This process is carried out during 48 hours at a temperature of -110°C and 1 Torr vacuum. Afterwards the materials are pulverized to a size of 500 µm and refrigerated until its use.

To produce 1 g of squid flour, 6 g of fresh squid are needed; and to obtain 1 g of krill flour, 7 g of fresh krill are required. At CENAIM we can produce 300 g/day of krill or squid flour. In the next table we show the biochemical analysis of fresh squid, squid and krill flour (liophilization):

TABLE 13. BIOCHEMICAL ANALYSIS EXPRESED IN DRY WEIGHT.

| Parameters | Fresh squid | Squid Frozen | Squid meal | Squid meal | Krill meal |
|---|---|---|---|---|---|
| Moisture | 76.65 | 80.76 | 7.58 | 7.97 | 16.85 |
| Lipids (%) | 21.32 | 21.31 | 7.61 | 8.70 | 15.40 |
| Σ n-3 (%) | 36.53 | 32.76 | 40.39 | 41.20 | 32.26 |
| Σ n-6 (%) | 8.33 | 14.42 | 5.82 | 3.59 | 3.52 |

The liophilization process maintains the squid biochemical characteristics as can been seen for the fatty acid methyl esters n-3 and n-6.

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# PROTEIN REQUIREMENTS IN ARTIFICIAL DIETS OF *PENAEUS VANNAMEI* JUVENILES

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## ABSTRACT

*Penaeus vannamei* juveniles, with an average weight of 0.93 g were fed with artificial diets. Measured protein levels of the five diets ranged from 25 to 45% in increments of approximately 5 %. After six weeks the highest level of growth was obtained with the diet containing 40% protein, while growth decreased at the highest protein level (45%).

The animals fed at the three lowest protein levels (25,30 and 35%) attained significantly ($P \leq 0.05$) lower weights (4.11,3.50 and 3.57g respectively) than those fed with diets containing 40 and 45 % of protein (6.57 and 6.52g). However the lowest levels of survival (68 and 77%) were observed with the highest protein level diets.

## KEY WORDS

Protein requirements,artificial diets,feed efficiency

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## INTRODUCTION

Penaeid shrimps are one of the most important and extensively cultured crustaceans throughout the world. This is due to its great demand and to its high market value. It is also ideal for intensive cultivation because of its adaptability to different culture systems, rapid growth, availability of seedstock through artificial propagation and positive response to supplemental feeding.

In Ecuador the development of shrimp culture can not be ignored and it now constitutes one of the most important source of hard currency through exportation. Nevertheless many problems still remain to be solved, particularily those concerning the quality and cost of diets.

The development of an economic artificial diet is an essential requisite for the successful rearing of penaeid prawns, and is seen as an essential requirement for the continued profitability of this industry.

As the cost of protein dominates the overall price of a finished diet, it is of great importance to identify the minimum quantity of protein for *P. vannamei*. As the quality of protein has also been shown to greatly affect growth performance. Due to both availability and cost considerations, the first attempts to provide balanced diets for shrimp in Ecuador were characterized by their high proportion of fish meal. However preliminary investigation soon indicated that fish meal could not provide all the dietary requirements for satisfactory growth and feed conversion (Colvin, 1976). Largely as a result of these findings, alternative diets incorporating high quality protein were prepared at the CENAIM Research Institute.

This study was designed to determine the optimun dietary requirement of protein for *P. vannamei* juveniles in order to achieve the best growth and survival.

## MATERIALS AND METHODS

The experiment was carried out from February to April of 1992, over a 63 days period .The test animals used in this assay came from wild nauplii populations which were reared in the laboratory to postlarval stages and ongrown farm ponds of the area of Palmar ( Península de Santa Elena-Ecuador). They were acclimated to laboratory conditions for three weeks

before starting the experiment. During this period, they received a commercial diet containing 35% protein at a rate of 15% of their biomass/day.

The 665 juveniles randomly selected had a 0.93 ± 0·05 g average weight. Approximately 225 animals were frozen for a later analysis, the rest of them (450) were randomly distributed in 15 fiberglass tanks (capacity of 2MT), initial density in each tank was ten animals per square meter, with the following rearing conditions water replacement was 300% per day, photoperiod was normal, and an aeration system was used to dissolved oxygen within acceptable levels.

Temperature (T), pH, salinity (s) and dissolved oxygen (DO) were measured daily. Weekly, the nitrites ($NO_2$) and ammonia ($NH_4$) concentrations were determined. Additionally within the experimental area, maximum and minimum environment temperature were also measured daily.

Five diets containing 25 to 45% protein were prepared with approximately 5% increments. The diets were formulated to contain all predicted essential nutrients in equal quantity except for the protein level to be tested. The diet formulation was done with locally available animal and plant ingredients, instead of using purified test diets. Animal/plant protein ratio (2:1) was utilized.

The study consisted of five treatments with three replications per treatment. The treatments were randomized. The shrimps in the tank were fed with experimental diets containing various levels of protein. The daily food requirement was calculated using the tank biomass and the conversion table proposed by Lovell (1989). The diets, were suministrated after Lovell (1989) in three doses per day, at 02,10 and 18 hours respectively.

Every morning, before feeding, faeces and other detritus in each tank were siphoned out. Excess feed was also removed, but no correction for dry weight of uneaten feed was made in calculation of approximate conversion rates. Diets were evaluated on the basis of growth and survival data after being fed with the test diets for a period of 42 days.

Every week individual animals were measured to the nearest 0.5 mm (rostrum-telson length), blotted and weighted to the nearest 0.01g to determine the weight gain and adjustment of feed allowances.

At the termination of the feeding trial,the shrimps from each treatment were pooled, stored in plastic bags and kept frozen at -20°C for one day and freeze dried for later analysis.

Biochemical analysis were performed on the five diets, shrimp juvenile, directly from the farm supplier and experimental animals. The biochemical composition study was done using the following methodology: crude protein (Kjeldahl method), lipids (Bligh and Dyer,1959), phosphorus (AOAC,1975), crude fiber (with a fiber determiner machine MRK FIBER-MATIC ), moisture (INEN,540-1980) from the Ecuadorian Standardization Institute, ash (INEN,544-1980), calcium (INEN 546-1980). All chemical determinations were done in triplicate and reported on a dry matter basis. The amount of carbohydrates was indirectly calculated by substracting the amount of the four components (crude protein, fat, fiber and ash) from the total dry weight of the diet.

The data corresponding to the physico-chemical parameters were compared with a t Student test. The rest of the data were statistically processed with ANOVA (Williams,1986). When differences were found between means, the Duncan's multiple range test (Miller et al.,1965) for separation was used and results were expressed at the 95% confidence level.

## RESULTS

### PHYSICO-CHEMICAL PARAMETERS

The physico-chemical parameter values measured throughout experiment are shown in Table I. There were no significant statistical differences ( $P \leq 0.05$ ) between the mean values of T, pH, DO and salinity of the tanks water with diets and the water entrance. Temperature, pH, DO and salinity has and average and a range of 28.9 $\pm$ 0.26 °C (27.8-29.5), 8.03 $\pm$ 0.17 (7.1-8.3), 6.19 $\pm$ 0.43 $mg.dm^{-3}$ (4.1-7.14), 34.85 $\pm$ 0.22 ups (33.5-35.0) respectively. These parameters were quite stable throughout the experiment. Ammonia and nitrite concentrations varied among the different diets. The ammonia levels fluctuated between detection limit and 5.27µM, the highest levels were found in diets 2 and 4. The nitrite concentration fluctuated between 0.02 and 0.44 µM and fell well within established tolerance limits for prawns (Wickins,1976).

The ambient temperature in the experimental area was 31.6 °C with a range between 29.0 and 35.0 °C for the maximum and 27.9 °C with a range between 26.8 and 29.0 °C for the minimum.

DIET COMPOSITION

Table II shows the percentage composition of the major protein sources of the 5 tested diets. Final protein levels were a little different from the original formulations but were correlated. Table III shows biochemical composition of the diets determined by analysis.

GROWTH AND SURVIVAL

The mean body weights of *P. vannamei* juveniles fed at various levels of dietary protein for a period of 42 days are shown in Fig.1. The average weight gains of shrimp receiving the various treatments are presented in Table IV. Shrimp fed with 40% protein had the highest weight gain (0.94g). This gain was significantly higher ( $P \leq 0.05$ ) than those fed with the other protein dietary levels, except those fed with the 45% protein. No significant differences ($P \leq 0.05$) were observed among the weight gains of shrimp fed with the three lowest protein dietary levels (25-35%). A reduction in weight was obtained from the group fed with 45% protein.

The average feed conversion ratios (FCR) were relatively high in all treatments and ranged from 2.30 to 3.32. The 40% protein diet provided a FCR value of 2.77 but was not significantly different ($P \leq 0.05$) from the 25 and 45% diets (Table IV )

The protein efficiency ratio (PER= g live weight gain/g protein eaten) was high for the five treatments (0.88-1.34). The 25% protein diet gave a significantly higher PER value than those of the other diets. The PER values of shrimps fed at 30-45% protein were essentially the same.

No significant differences ( $P \leq 0.05$ ) were observed among the survival rates of shrimps fed with the three lowest protein diets. Shrimps fed with 40% protein content had the lowest survival rate (68%) but this was not significantly different from those fed with 45% protein content.

## DISCUSSION

Shrimp fed the 40% protein diet exhibited the highest weekly weight gain (0.94g). A similar results (0.99g) were obtained by Alava and Lim (1983) with *P. monodon* juveniles.

Comparison of the protein requirements of penaeid shrimps showed that they vary for different species. For *P.setiferus* a 28-32% protein requirement was reported (Andrews et al.,1972). For *P. aztecus* (Venkataramiah et al.,1975) and *P. monodon* ( Lee,1971) showed an optimun about 40%, while in *P. indicus* a diet containing 43% protein gave the best growth (Colvin,1976). *P. merguiensis* required 34-42% (Sedgwick,1979) while *Palaemon serratus* required 40% ( Forster and Beard,1973). The protein requirements of *P. californiensis, P.stylirostris* and *P. vannamei* postlarvae were similar and varied from 30 to 35% (Colvin and Brand,1977). *P. japonicus* was found to grow best when fed more than 60 % protein (based upon descriptions of diets tested by Deshimaru and Shigeno,1972) and at least 50% purified protein (Deshimaru and Kuroki,1975). Our results are in general agreement with the studies on *P. aztecus* and *P. monodon*. Differences in protein intake may be associated with species differences in feeding habits.

Growth of *P. vannamei* juveniles in the present study (137mg/day) was equal to that reported for similar sized penaeids fed with artificial diets in tanks. Fenucci et al., (1980) reported a 130 mg/day increase by *P. stylirostris* fed with a shrimp meal based diet, and Sick et al., (1972) reported a 140 mg/day increase for *P. aztecus* , also fed with a fish meal based diet.

The growth of shrimps however, is not only affected by the dietary protein quantity but also by its quality. Diets containing a mixture of two or more protein source are better utilized by shrimps than those containing a single protein source (Alava and Lim,1983).

The quality of diet require to achieve maximun growth also appears to be size dependent (Chen et al.,1985); smaller shrimps being more dependent than larger ones on animal protein content. In our trial the relationship between animal/vegetable protein was 2:1. The animal protein sources were shrimp head meal, fish and squid meals and vegetable protein sources were wheat bran, wheat and soybean meals. In these conditions it was obtained the best growth with the 40% protein diet during six weeks, starting with an average weight of 0.93 g.

Sedgwick (1979) found that the optimal utilization of protein by *P. merguiensis* was closely related to the energetic value of the diet and that the carbohydrate and lipid can also increase growth efficiency at suboptimal protein levels. The results in the present trial are in agreement with above mentioned statement. In our case the shrimp fed with suboptimal levels of protein (25% protein diet) showed the best protein utilization rate. As the level of the protein in the diet increases beyond the maximum requirement, the excess is stored as fat or carbohydrate. At lower levels of protein intake, if the diet does not contain enough energy, the most of the protein will be used for energy rather than for growth.

In penaeid shrimps fed with various protein level diets the survival rates decreased as protein levels increase (Andrews et al.,1972). This is in agreement with our data; the shrimps fed with 40-45% protein content had the lowest survival rate than those fed with the three lowest dietary protein levels.

Under the conditions of this experiment, the following conclusions appear to be justified:

- Results of this trial indicated that 40% was the optimal protein dietary level with respect to growth for juveniles of *P. vannamei*.

- Protein levels above 40% produced a decrease in growth.

-Juveniles of *P. vannamei* fed with the three lowest protein diets had final mean body weights which were only about half of the mean weight obtained with 40% protein diet.

-Food conversion rate (FCR) obtained with 40% diet was not different from the FCR obtained using 25 and 45% protein level.

-Protein efficiency ratio (PER) was the same in all diets except for the 25% protein level, which gave the best result.

Survival rates decreased slightly in groups with the higher protein levels.

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# Activities Report " Mollusc culture Project"

INTRODUCTION

Mollusc culture project is part of the fish-mollusc culture project under the Technical Cooperation Program existing between CENAIM and JICA . The objective of this project is to develop and/or adapt techniques for seed production and grow out culture of commercially important bivalve molluscs.

Specie selection criteria were based on the biology, market potential, availability of broodstock and existing culture techniques for the specie or similar species. The following specie were selected for the development of the project.

Rock oyster (*Crassostrea iridisens*)-Native
Pacific or japanese oyster (*Crassostrea gigas* )- Imported
Pacific calico scallop (*Argopecten circularis* )-Native

Fom August 1990 to July 1992, activities within the following topics were carried out:

1) SPECIES SELECTION
2) SPAWNING (EGG COLLECTION)
3) SEED PRODUCTION
4) REARING MANAGEMENT
5) WATER QUALITY MONITORING IN CULTURE AREAS

These activities are described bellow:

## 1.0 SPECIES SELECTION

### 1.1 NATURAL POPULATION EVALUATION

The equipment needed to evaluate natural mollusc population did not arrive on time, therefore, it was not possible to carry out this activity as planned. Two diving trips were made to determine the existing species in the waters surrounding CENAIM. As a result of those trips, pictures of collected specimen are showed in the publication called "A FIELD GUIDE TO THE EDIBLE FISHES AND SHELLFISHES IN COASTAL WATERS OF ECUADOR" (1992).

On May 7th, 1992 the equipment arrived (2 dredges ). Four sampling trips were done three nautic miles off Playas (July 8th, 15th, 22th, and August 6th) to determine the localization of scallops banks. We could

not locate the banks but we were able to collect and bring to the laboratory about 500 animals. Once in the tanks, survival was monitored daily and at this moment (August 21, 1992) we have 179 scallops alive with an average shell length 41mm and a mean weight of 24.5 g .

The evaluation of natural populations of native molluscs will continue on September 1992.

### 1.2 INTRODUCTION OF NON NATIVE SPECIES

The first introduction took place on March 8th,1991 when 30.000 spats of *Crassostrea gigas* with an average shell length of 9.18 mm were imported from the Universidad Católica del Norte in Coquimbo, Chile. The spats, upon their arrival, were placed in trays at a density of 5500 spats/tray and placed in 900 l tanks with a flow rate of 10 l/min. They were transfered to the culture areas after a maximun of 66 days.

On November 19, 1991; a second group of 10.000 seeds of C. *gigas* were imported from the same University in Chile. The seed had an average shell length of 26 mm and mean weight of 1.35g. After their arrival and before their transfer to the culture systems, the oysters were placed in trays at a density of 2000 seed/tray.

### 1.3 CONDITIONIG OF BROODSTOCK OF NATIVE AND INTRODUCED SPECIES

For purpose of this work, conditioning means gonad development and maturation. In nature this processes are under several exogenous and endogenous factors which have to be simulated in the laboratory. Since temperature, food abundance and availability are the most important factors for the species under consideration, broodstock are kept in tanks with water at 20°C and fed continuosly with micro-algae (*Chaetoceros* sp and *Isochrysis* sp) maintaining a concentration of 100,000 cells per ml in the culture water.

Scallops:

Three conditioning trials were performed. For the first trial, adult scallops were brough to the Laboratory on December 1990 and maintained at 20°C on temperature controled tanks until November 1991. During this period scallops were fed daily with *Chaetoceros sp.* (50,000 cells/ml) and spawning occured three times.

To conduct the second trial, aproximatelly 500 individuals with the following characteristics were purchased in Playas on Sep. 10th,1991:

- shell length: 40 to 60 mm.
- individual weight:16 to 50 g

They were inmediatelly transported to the laboratory and mantained in tanks for five days. After this period, the selected organisms were placed in one experimental pond located at A. Fuentes shrimp farm in Palmar

until January 29th/92. They were returned to the laboratory to continue with the conditioning process prior spawning and larvae production.

The third trial was conducted with scallops brought from the shrimp farm during February 1992. Eighty (80) broodstock scallops were maintened in controlled temperature systems (17-21 °C) and fed with microalgae *Chaetoceros* sp. (500,000 cell/l) and *Isochrysis galvana* (1'000.000 cell/l) at a feeding rate of 140 L during this period.

Oyster:

To start the conditioning process with the Pacific oyster (C. *gigas* ), in January 28th 1992 one hundred broodstock with shell length and individuail weight varing from 40 to 72 mm and 40.1 to 152.6 g. respectivelly were transported from the shrimp farm to the laboratory.
Since C. *gigas* is not a native specie, the broodstock was the result of the culture trial performend in the A. Fuentes shimp farm with the seed imported from Chile on March 1991.

On February 11, they were placed in controlled temperature systems (16.5-25.5°C) and fed with microalgae *Chaetoceros* sp and *Isochrysis galvana* at feeding rate of 350 L. Five (5) organisms were sampled weekly in order to follow gonadal development of the broodstock.
A second conditioning trial started on May 19, 1992 when 160 adult oyster were placed in two temperature controled tanks and kept at 18 °C. To promote maturation, oysters have been overfed with *Chaetoceros sp., Isochrysis sp., Tetraselmis sp.* and corn starch.

## 2.0 SPAWNING(EGG COLLECTION)

Scallop:

The scallop from the first group spawned naturally three times on April 16 and 30 and in June 19 and September 18, 1991. One day after their spawnig, the larvae produced were siphoned and transfered to bigger tanks for their culture.

Selected individuals from the second and third group were forced to spawn by means of increasing the water temperature and over feeding. This procedure was carried out on February 5 and 13, 1992.

Oysters:

During February 1992 oyster broodstock collected from the shrimp farm were opened to check their maturity. Mature broodstock (males and females) were forced to spawn by reaping their gonads. Next spawnings were registred during February 20 and March 28

The same procedure was done in April 3 and June 10,1992 after observing mature broodstock in the culture tanks.

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## 3.0 SEED PRODUCTION

### 3.1 micro algae culture

Since the size of the micro algae currently used for the culture of shrimp larvae is to large for mollusc larvae, it was necesary to import micro algae seed from Chile and Japan.
The following species were introduced:

| SPECIE | COUNTRY OF ORIGIN | DATE OF INTRODUCTION |
|---|---|---|
| *Isochrysis* sp. | Chile | March 1991 |
| *Pavlova lutheri* | Japan | April 1991, January 1992 |

### 3.2 LARVAE CULTURE (NATIVE AND INTRODUCED SPECIES)

Scallops:

Due to the fact that not previous experience existed in the country about scallop production, the spawnig of April 17 and 30, 1991 are considered as the first experience. The eggs produced were fertilized and the larvae culture was carried out only to understand the development of the larvae of *A. circularis*. The results are shown in the Fig. 1

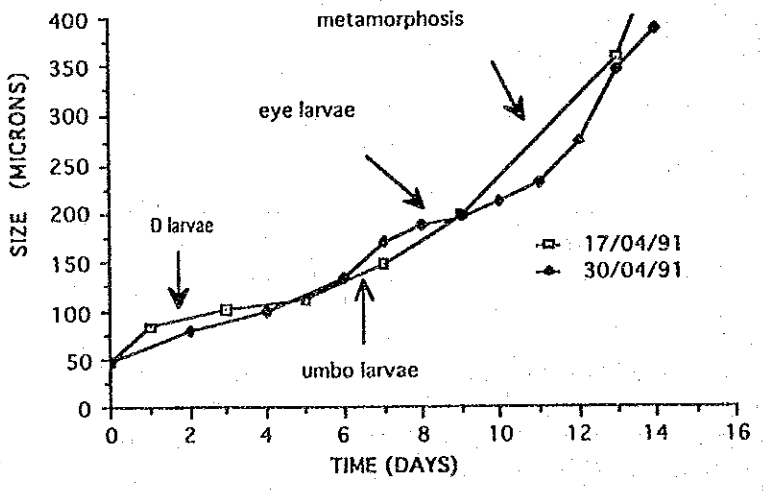


Fig. 1 Larvae stages of the Calico Scallop (*A. circularis*)

For the second group, the first spawning ocurred on February 5, 1992; produced very few eggs and the rate of fertilization was very low

about 60,000 larvae were obtained. Mortality was 100% ten days after spawning due to a bacterial out-break.

From the spawning occurred on February 13, 1992, 3500 seeds with a mean shell length of 10 mm were obtained under controlled conditions. Water temperature in the culture tanks ranged between 27 and 30°C.

The following table shows the date of spawning, average temperature in the culture tanks and time required by the larvae to reach metamorphosis.

| Date | Average Temp. °C | Time (Days) |
|---|---|---|
| April 16 | 28.0 | 15 |
| April 30 | 28.5 | 12 |
| June 19 | 29.0 | 17 |
| September 8 | 25.0 | 18 |
| February 5 | 28.0 | ----- |
| February 13 | 28.3 | 19 |

The number of scallop seeds produced in each spawn are shown in the following table.

| Spawn | Date | No. Seeds |
|---|---|---|
| First | April 16 | 15.000(A,B) |
| Second | April 30 | 11.000(A,B) |
| Third | June 19 | 17.000 (A) |
| Fourth | September 18 | 15.500 (B) |
| Fifth | February 5 | 0 |
| Sixth | February 13 | 13.500(A,B) |

A) Atached to sun shield nets
B) Directly attached to tanks.

Oyster

From the first spawning occured during February 20, aproximately 200.000 oyster larvae D were produced with a mean size of 80 microns. From this group 21,230 larvae settled and were collected as seed. From the second spawning (Feb. 28) aproximately 6,200 seeds were obtained. The growth curve for larvae oysters is shown in Fig. 2.

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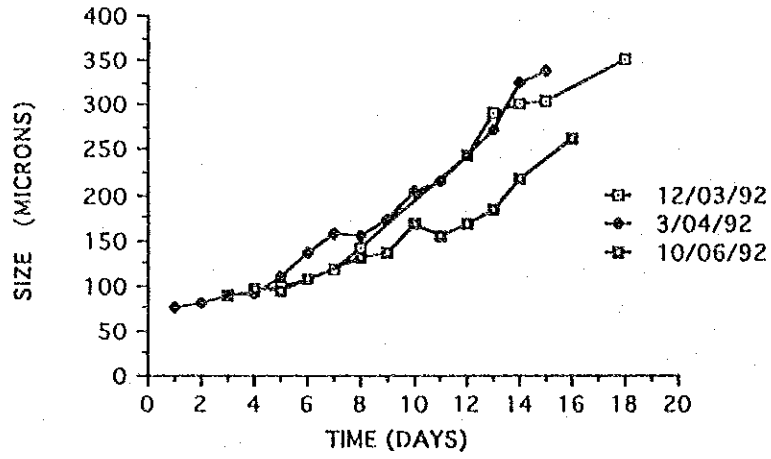


Fig. 2 Growth of larvae oyster

The following table shows the date of spawning, the average water temperature at culture tanks and the period took by the larvaes to metamorphoce :

| Date | Average Temp. °C | Time (Days) |
|---|---|---|
| February 20 | 28.4 | 19 |
| March12 | 28.8 | 18 |
| April 3 | 29.2 | 15 |
| June 10 | 27.2 | 16 |

Number of oyster larvae produced in each spawning and final seeds collected are shown in the following table.

| Spawn | Date | No. Larvae D | No. Seeds(*) |
|---|---|---|---|
| First | February 20 | 200,000 | 21,230(A) |
| Second | March 12 | 100,000 | 6,200(B) |
| Third | April 3 | 4'130,000 | 150,000(C) |
| Fourth | June 10 | 270,000 | 486(B) |

*The following substrates were use to collect the oysters at the end of their larval cicle.
(A) Colectors made of entire shells of *Argopecten purpuratus*
(B) Grinded shell (<350 and >180 microns) of *C.gigas.*

(C) Acetate sheets, fiber glass sheets, grinded shell, triturate and entire shell of C. *gigas* in bags.

It is important to notice that the best collection took place in colectors made out of entire shells while the worst was observed in fiber glass sheets.

## 4.0 GROW OUT MANAGEMENT

### 4.1 Seed aclimation

Because of the difference in water temperature between Chile and Ecuador, the oyster seed were acclimatized during 2 hours upon their arrival to CENAIM. After this period the seed were kept under observation during 20 days in order to determine their survival. At the end of this period total mortality was 4% for both shipments.

### 4.2 Experimental culture of native and introduced specie

Oyster

After the period of aclimation, the seed imported from Chile in 1991 was cultured from April 1991 through February 1992. The culture trials were conducted in the ocean and in a pond in combination with shrimp.
The second group of seeds was stocked for the first time in a shrimp pond on December 20, 1991and after that on March 11, 1992.
The culture techniques as well as the results of the culture are described below.

#### 4.2.1 culture in the ocean

*METHODOLOGY*

Oyster seed were placed in pearl-nets hanging from a 200 m Long-line instaled 1000m off the shore in front of CENAIM. The depth at this point is 11m at low tide.

On march 28, 11000 juvenile oysters were stocked and 3,500 on April 14. A mollusc culture in an estuary was considered but logistic problems and the lack of culture systems restrained the culture to two areas (ocean and shrimp farm). The oyster seed allocated for culture in the estuary was divided to the other areas in consideration.
The following are the characteristics of the seed at stocking time.

FIRST STOCKING
mean shell high: 13.6 mm.
mean weight: 0.154 g

SECOND STOCKING
mean shell high: 21.3 mm.
mean weight: 0.494 g

Oysters were checked avery 14 days to determine the growth rate, survival as well as to clean the culture systems. A record of salinity, temperature, DO, Turbidity and pH was kept through the culture period. Bacteriological analysis from oysters and fitoplankton analysis from the culture water were performed monthly.

RESULTS

The culture period lasted 217 days for the system in the open ocean, and Fig. 3 shows the growth rate for the oysters at the three densities per pearl-net (125, 250 and 500), considered for the study.

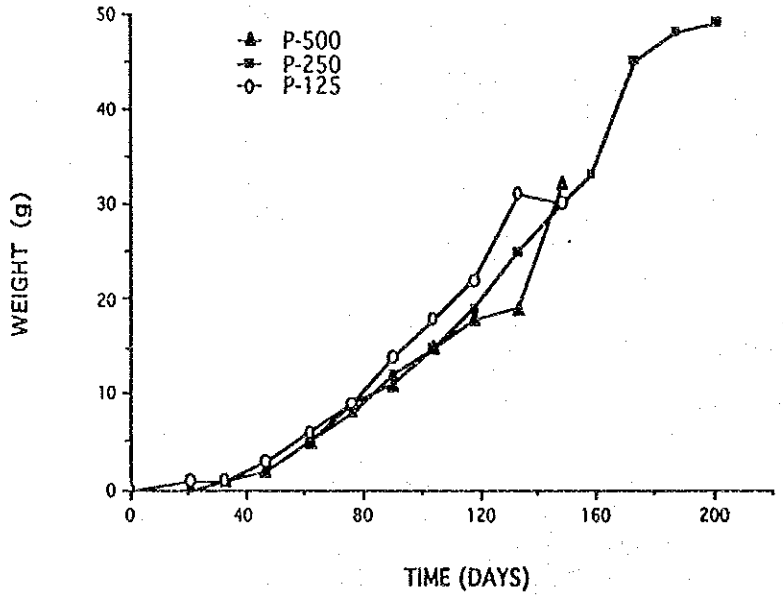


Fig. 3 growth of (*C. gigas*) in the ocean

From the figure we can see that growth increase after 30 days and after 160 days the oysters reach 35 g. After August 24 there is not data available for the oyster cultured at 125 and 500 per pearl-net because they all die due to predation.

By the end of the experiment, oysters cultured at a density of 250/pearl-net reach a mean weight of 45.19 g and a mean shell height of 68.48 mm. Oysters cultured at densities of 125 and 500/pearl-net had the

following characteristics during the last sampling: mean weight of 30 and 32 g and a mean shell height of 65 and 66 mm respectively.

Survival in the Ocean for all the groups of oysters was null as it is shown in Fig 4. Mortality was caused mainly due to the action of predators even thought some poaching occured.

Salinity varied from 34 to 36 ppt and water temperature varied from 22.7 to 28.8 °C in the surface and from 22.4 to 27.6 °C at a depth of five meters.

In order to continue with the oyster culture in the ocean, a Long-line system, (100 m length) was installed again on May, 29th of 1992 in front of CENAIM at 200 m from the beach, the depth at low tide is 16 m. On June 4th, 1992; 712 juvenile oysters were placed on the system.

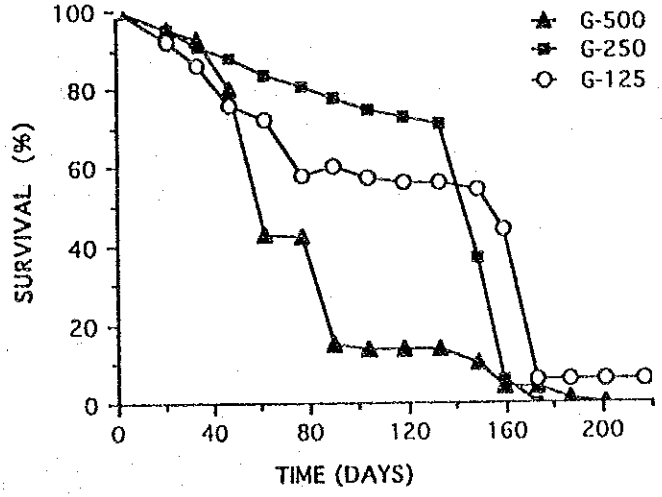


Fig. 4 Survival of oysters cultured in the ocean

4.2.2 pond culture

Oysters were culture in combination with shrimp in a 0.1 Ha pond at the Alfredo Fuentes shrimp farm located near Palmar (10 min south of CENAIM).

*METHODOLOGY*

The oysters were cultured using three different systems:

a) Pearl-nets with a surface area of 0.1 m2 which were placed in 11 ropes. Each one was suspended by the end by poles buried in the ground and placed inside the pond.

b) Trays composed by 9 x 12 mm plastic mesh surrounded by a wood frame. The surface area of this system was 0.6 m2.
c) Pillows with a surface area of 0.5 m2 made with 9 x 12 mm plastic mesh.

Pillows and trays were placed in a bamboo structure which kept those culture systems 0.6m above the bottom.

On April 17 and May 14, 11,000 and 3,500 seeds were stocked, respectively. Oysters were placed in pearl-nets at densities of 250 and 125 and had the following characteristics at stocking time

FIRST STOCKING
mean shell high: 14 mm.
mean weight: 0.243 g
SECOND STOCKING
mean shell high: 21.3 mm.
mean weight: 0.494 g

When the oysters reached 10 g (after 90 days) 75% of them were transfered to pillows and trays at densities of 450 and 600 respectively. The density in the trays was reduced by half before the end of the culture.

On April 10 and 11 1991, 5.000 post-larvae of *Penaeus vannamei*, cultured in CENAIM, with a mean individual weight of 0.06 g were stocked in the pond.

*METHODOLOGY*

The integrated culture was sampled every 14 days to determine the growth rate for the oyster and shrimp, the survival of the oysters and to clean the culture systems as well. A record of salinity, temperature, DO, Turbidity and pH was kept through the culture period.

Bacteriological analysis from oysters and shrimp as well as fitoplankton analysis from the culture water were performed monthly.

To increase the amount of fitoplankton in the culture water, fertilization was applied periodically at a rate of 30 kg of urea per Ha. Shrimp were fed with artificial feed after they reach a mean individual weight of 8g. Shrimp and oysters were harvested on November 5 and 6.

*RESULTS*

Fig.5 shows the growth of oysters on pearl-nets for the two densities considered (125 and 250 per pearl-nets) and it can be noted that for the first 50 days, growth was not correlated to the culture density.

After 211 days of culture , the oysters cultured at the density of 125 per pearl-net reach a mean shell high of 90mm and a mean individual

weight of 94.33g while the ones cultured at the density of 250 per pearl-net reach a mean shell high of 92mm and a mean individual weight of 82.5g.

The growth curve for oysters cultured on trays and pillows is shown in Fig.6 very little difference in growth exist between the two cultured systems. At harvest, the oysters cultured in trays reach a mean shell high of 97.1mm and a mean individual weight of 75g while the ones cultured in pillows reach a mean shell high of 101mm and a mean individual weight of 73g.

Survival for the oysters cultured in the shrimp pond was more than 90% for the three culture systems under consideration. The low mortality was due to the fact that the pond is a controled system were no predators and competitors exist.

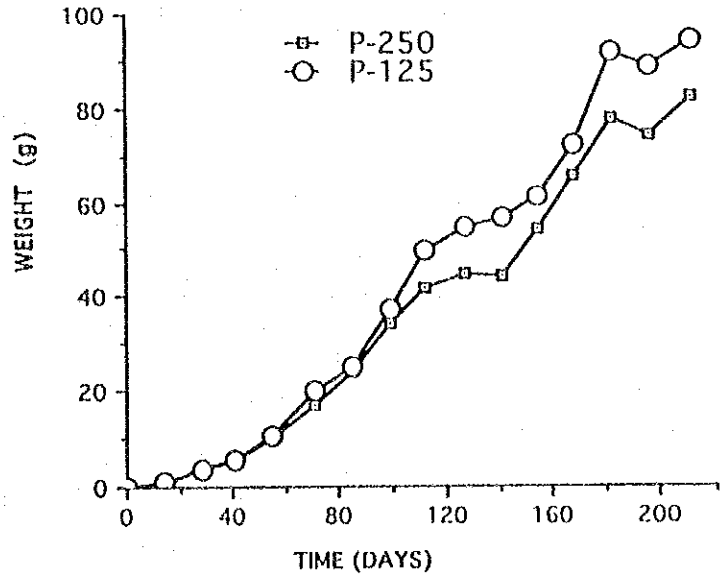


Fig.5 Growth of oysters in pearl-nets

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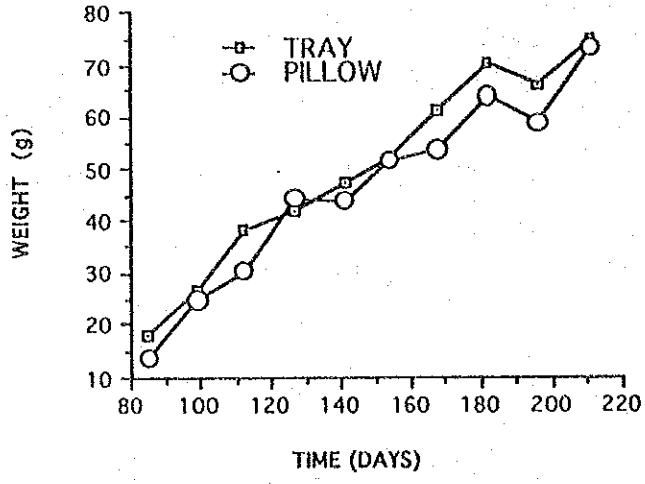


Fig. 6 Oysters growth cultured in trays and pillows

Shrimp and oysters were cultured during 211 days, Fig. 7 shows shrimp growth. When the shrimp reached 8 g, growth stooped and shrimp was fed, using commercial diet. On November 6 and 7; 3,784 shrimps with a mean individual weight of 14.8 g and mean total lenght of 129 mm were harvest; this is equivalent to a production of 1233 lb/Ha.

Survival at harvest was 75.7% and during the culture period, shrimp were periodically analysised to determine gregarines. Apparently they do not affect the shrimp growth rate.

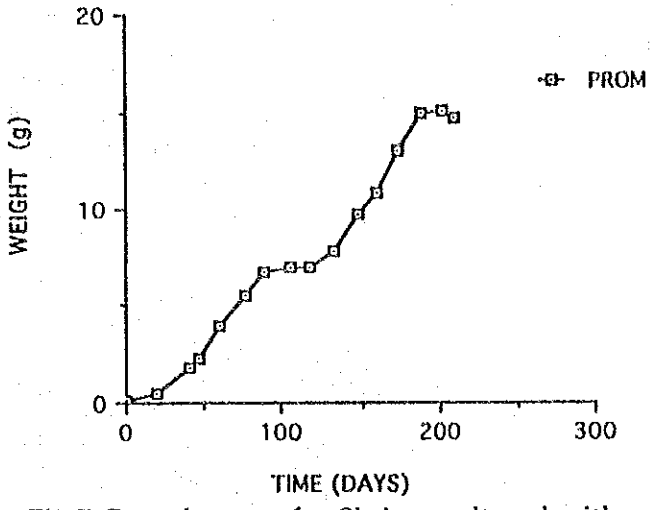


Fig.7 Growth curve for Shrimp cultured with oysters.

Water temperature and salinity in the pond water varied from 35 to 39 ppt and from 23 to 29.8 respectively.

A second trial of oyster culture in shrimp pond were carried out during March 11, 1992. Pearl nets with oyster were placed into a drainage chanel and experimental pond, in order to begin a new grow out experience. However, heavy rainfall and flood caused by "EL Niño", as well as sedimentation due to collapsing of channel walls causing the burying of the culture system produced a 50% oyster mortality. Due to this problem, the remaining oysters were brought to the laboratory.

At the end of May salinity levels in the shrimp pond increase to 27 ppt. The oysters kept in laboratory were returned again to the experimental pond

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4.2.3 culture of scallop (*A. circularis* )

METHODOLOGY

An experimental culture of scallop was carried out using seed produced in the laboratory. The objective of this culture was to have an idea of the performance of this specie under culture conditions in the ocean and in one pond in combination with shrimp and oysters.

Scallops with a mean shell high of 3.5 to 5.6mm were placed on both locations using pearl-nets as culture systems. The initial data for the scallops is shown bellow:

| | Ocean | Shrimp Pond | Shrimp Pond |
|---|---|---|---|
| Date of stocking | 28/08/91 | 11/06/91 | 29/08/91 |
| # of scallops | 4000 | 2100 | 14000 |
| weight (g) | 0.014 | 0.014 | 0.038 |
| shell high (mm) | 3.7 | 3.5 | 5.6 |

Due to the lack of culture systems , density was not taked under consideration because it depended on the availability of pearl-nets at the time of stocking.

For the 14,000 scallops stocked in the shrimp pond on August 29, it was necessary to construct pearl-nets using material locally available (Polimalla 1mm).

In the ocean the scallops were cultured during 162 days and by the end of this period they reached a mean individual weight of 23.4 g. The ones in the shrimp farm were cultured during 141 days and at the end of this period reached a mean individual shell height of 35.2 mm and a mean individual weight of 14.8g. as shown in Figures 8.

It is important to point out that the scallops stocked on August 29 died because the mesh used in the construction of local pearl-nets was cloged with algae and organic matter.

After this experience a second group of scallops from the spawn ocurred in Febrary 13/92 were placed in experimental shrimp pond. Unfortunately due to heavy rainfall the salinity decreases down to 5 ppt. All the scallops cultured in the system died.

From the preliminary results obtained in this experiment we can say that the native scallop has potential for the culture in the ocean and in combination with shrimp. At this moment we have enough pearl-nets to conduct new culture trials in which more data will be collected.

*CONCLUSIONS*

- From the data obtained we can conclude that is possible the culture of the Japanese oyster in our country. This open the possibility for the diversification of the aquaculture in Ecuador.

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-The polyculture oyster-shrimp seems to have a very big potential, for this reason is necesary to conduct more trials in order to solve the potential problems that could arise from this culture.
-It is necessary to conduct new culture trials in the ocean in order to determine the best technology for our conditions and to define ways to control predators and competitors.

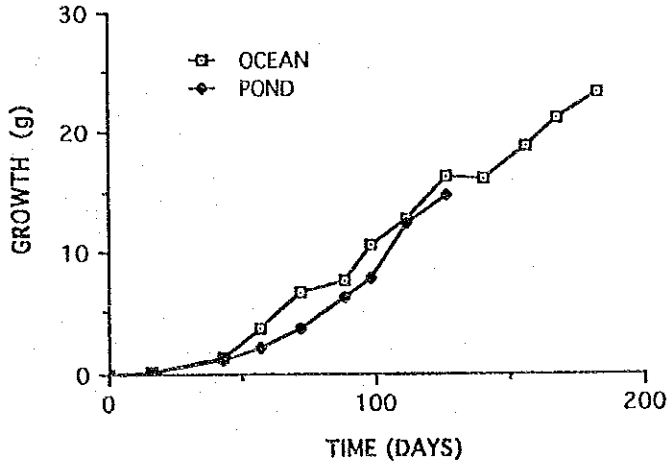


Fig. 8 Growth of the calico scallop

-Based on the first experience we believe that *Argopecten circularis* seed can be culture in the laboratory and the grow out could be carried out in the ocean as well as in shrimp ponds.
-It is necesary to have a reliable source of mollusc seed and for this reason is important to continue working in the laboratory in order to master this technology.

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# POLYCULTURE OF THE PACIFIC OYSTER (*Crassostrea gigas*) AND THE WHITE SHRIMP (*Penaeus vannamei*) IN ECUADOR.

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**Experto JICA (Japan International Cooperation Agency) en cultivo de Moluscos

## Abstract

This report presents part of the information obtained in the Project entitled "Fish and Mollusc Culture" carried out by the National Aquaculture and Marine Research Center (CENAIM) with the cooperation of the Japan International Cooperation Agency (JICA).
In order to determine the feasibility to develop an oyster-shrimp polyculture in Ecuador, 28000 seeds of the Pacific oyster, (*Crassostrea gigas*,) and 5000 post-larvae of the white shrimp (*Penaeus vannamei*) were stocked in a 0.1 Ha. pond located in Palmar at the Guayas Province on April 13th, 1991. The study lasted 211 days and at the stocking time the oysters had a mean length of 14 mm while the shrimp had a mean weight of 0.06 g. At the end of the culture period 560 kg/Ha of shrimp and 240.000 oyster/ha were harvested. The mean weight and length reach by the organisms were 84 g and 95 mm for the oysters and 15 g and 13 mm for the shrimp with a total survival of 86 and 76 % respectively. Microbiological analysis showed absence of coliforms in the oysters. There was an sporadic occurrence of gregarines in the shrimp which suggest that its presence is not influenced by the polyculture with oysters.

## Resumen

Este Informe presenta parte de la información obtenida en el Proyecto "Cultivo de Peces y Moluscos" desarrollado en el Centro Nacional de Acuicultura e Investigaciones Marinas (CENAIM) con la colaboración de la Agencia Internacional de Desarrollo del Japón (JICA).
Con el objeto de determinar la factibilidad del policultivo ostras-camarón, el 13 de Abril de 1991 se sembraron 28000 semillas de ostra del Pacífico, *Crassostrea gigas*, (Thunberg, 1975), y 5000 post-larvas de camarón de la especie *Penaeus vannamei* en una piscina camaronera de 0.1 Ha. localizada en la zona de Palmar en la Provincia del Guayas. Al momento de la siembra las ostras tenían una longitud promedio de 14 mm y los camarones un peso de 0,06g. Despues de un período de cultivo de 211 días se cosecharon 560kg/ha de camarón y 240.000 ostras/ha, El peso y tamaño promedio alcanzado por los organismos fue de 84 g y 95 mm para las ostras y de 15 g y

## Introduction

Polyculture is not a new concept in aquaculture, it has been practiced for many years in Asia and the simplest form of polyculture is the byculture (integrate culture or the culture of two species in the same pond or system) to which we will refer in this paper with the culture oysters-shrimp.

Previous research carried out in different parts of the world (Manzi (1989, 1990), Osorio (1989), Wang (1990,1991)) demonstrated the feasibility to develop this byculture. In economics terms, this could be an attractive alternative since the culture of other commercially important organism (oyster) within the same infrastructure used to culture shrimp would help to reduce the production costs and to optimize the use of the shrimp farms.

The following are the principal characteristics that make this polyculture possible:

a)Oysters like shrimp are organisms that live and develop in brackish (estuaries) and salt (ocean) environments having for this reason similar water quality requirements.

b)Both species can be cultured within the same pond because the oysters are static organisms that need to be placed in special culture systems for them to growth. The use of this systems is good for the shrimp because will provide with cover during the molting stage and will increase the substratum were the shrimp can seek for food (Manzi 1889, Wang 1990).

c)Since molluscs compared with shrimp are located in a lower level in the food chain there is not competition for food between the two organisms . Oysters will feed on the phytoplankton and in the dissolved organic matter which are present in the pond water due to the fertilization and as a left over of the artificial food currently suminis

d)The feces and pseudofeces expeled by oyster, which have a micropellet appearance and are composed mainly by non digested microalgaes, can be used by the shrimp as a supplementary source of food.

e)Oxygen concentration in the pond is not affected by this byculture because oysters eliminate algae and organic matter, which are oxygen consumers, through feeding (Wang, 1990). This has been demonstrate in the past by Cassinell (1979) who reported that in a shrimp pond, algae, not shrimp, is the mayor consumer of oxygen and by Boyd who reported in 1989, that in an intensive catfish culture, 75% of the total oxygen consumption was due to algae, detritus and microorganisms present in the water.

In the other hand, the high capacity of molluscs to filtrate water (according to Jorgensen (1952), a 50 g oyster is able to filtrate 78 liters of water each 24 hours) produces a circulation in the pond which is equivalent to an increase in the pond's water exchange rate. Wang (1990,1991) reported that 150.000 oysters placed in a 1ha pond will filtrate 12.000 $m^3$ which is equivalent to a water exchange of 120% per day and four times the daily water exchange recommended for an intensive shrimp culture system.

Materials and Methods

In order to determine the feasibility of culturing molluscs in combination with shrimp, 28.000 spats of the Pacific Oyster (*Crassostrea gigas* ) and 5.000 post-larvae of white shrimp (*Penaeus vannamei,*) were stocked in a 0.1ha pond located in Palmar, in the Guayas Province.

Oysters used in this study were part of a group of 60.000 spats imported from Chile in March 1991 in order to carry out mollusc culture research at the National Aquaculture an Marine Research Center (CENAIM). Before been stocked in the pond, the oysters were depurated and acclimatized in the laboratory during forty days. Shrimp post-larvae was stocked directly in the pond and were produced at CENAIM's shrimp larvae culture laboratory starting from maturation nauplii.

Shrimp and oysters with a mean weight of 0.06 and 0.3 g respectively were stocked on April 11 and 17, 1991. Oysters were placed on 56 pearl-nets at a density of 500 organisms per system and when they reached 10 g were transfered to trays and pillows at densities of 600 and 450 per system respectively. After 90 days and because of the high growth rate observed by the oysters in the trays, the density in those systems were reduced by half (300 oysters per tray) and maintained that way trough the rest of the study. In order to maintain the uniformity during the study, 10 culture systems, from which samples were took, were marked and placed randomly in the pond,

The following are the main characteristics of the culture systems used in this study:

a)"Pearl-nets": Are systems currently used in Japan to culture pearl oysters, they have an area of 0.1 $m^2$, pyramidal in shape and are made of plastic mesh. Pearl-nets used in this study were suspended from ropes installed along the pond.

b)"Trays": Made with rigid plastic mesh attached to a wood frame. They had and area of 0.6 $m^2$ and the mesh used had a light of 9 x 12 mm.

c)"Pillows": Made out plastic mesh (9 x 12 mm. light) with an area of 0.5 $m^2$.

Throughout the culture period temperature and oxygen were measured using an YSI-57 oxigenometer. pH was determined using a pHmeter Central Kagaku UC-23.

In order to determine the growth rate and mortality of the oysters, morphometric measures (shell length, high and wide), total weight and survival were recorded twice a month for 260 oysters from the control systems.

Shrimp growth was determined sampling 20 individuals twice a month. Hephatopancreas and intestine samples from 10 individuals were examinated monthly in order to determine the presence of gregarines.

Gregarine analysis as well as microbiological monitoring in order to determine the presence of *vibrio sp* and *coliforms* were performed monthly at microbiology and Pathology lab at CENAIM.

Finally, a qualitative and quantitative analysis of phytoplanckton in the culture water at the pond was performed monthly .

## Results and Discussion

During the culture period, salinity fluctuated between 35 and 39 ppt; temperature ranged between 23 to 28 °C; pH varied from 7,8 to 8,6 and dissolved oxygen was maintained within the permissible range (>4mg/l). Temporal fluctuation of those parameters is shown on Figs 1 and 2.

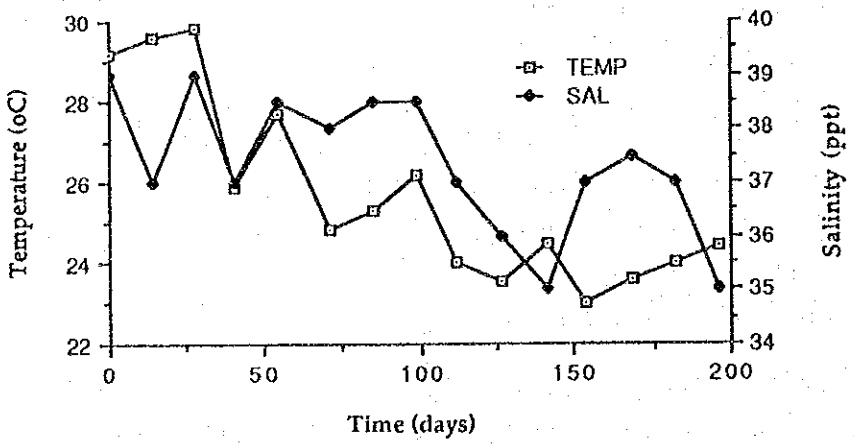


Fig. 1 Water temperature (°C) and salinity (ppt) variation within the pond during the culture period. (April-Octobrer 1991).

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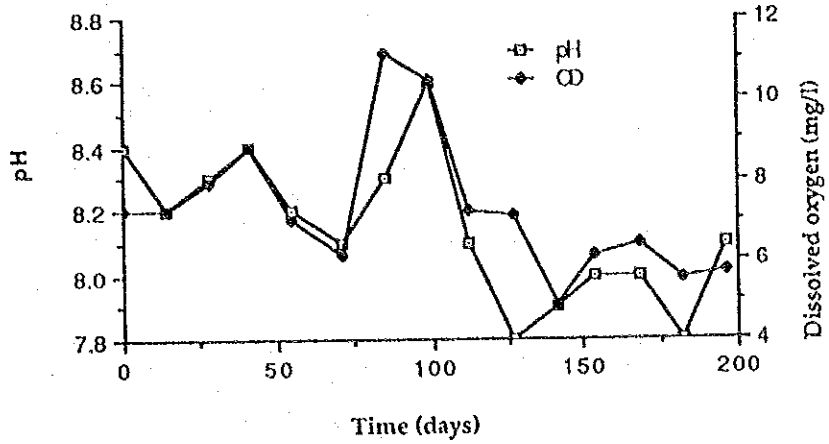


Fig. 2 pH and dissolve oxygen (mg/l) variation within the pond (April-October, 1991)

Post larvae shrimp was stocked directly in to the pond and its growth could be described in two phases (Fig 3). During the first phase which lasted 90 days, shrimp was not feed on artificial diet and shown a stable growth rate of 0,5 g per week until they reach 7 g when the growth stoped for about 30 days. The second phase started immediately after we add artificial diet to the pond and growth restarted at a rate of 0,6 g per week and lasted until shrimp reach 15 g at the end of the study. We believe that the lack of growth observed at the end of phase one is probably due to the need of animal protein (present as fish meal in the artificial diet) in stead of vegetal protein (present in the pond as phytoplankton and molluscs feces which was the only food source during the first phase) by the juvenile shrimp.

At the end of the study 56kg of shrimp equivalent to a production of 560 kg/ha were harvested. Total survival was 76% which is very good if we consider that in Ecuador survival for shrimp stocked directly at the density used in this study varies from 45 to 60%

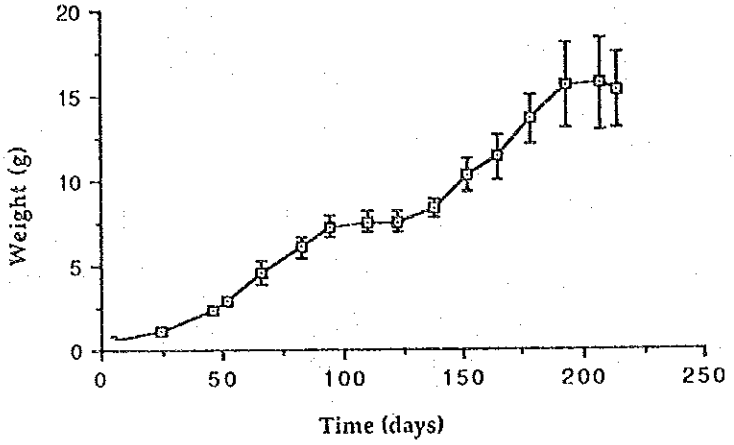


Fig. 3. Growth curve for the withe shrimp (*Penaeus vannamei,*) culture in combination with the Pacific oyster (*Crassostrea gigas.* ) (95% Conf.Int.)

Contrary to shrimp, oysters shown an stable growth rate throughout the study reaching an mean individual weight of 84 g after 211 days of culture. Figure 4 shows their growth curve and we can observe that oysters reached the minimal commercial size (60 g) in approximately 150 days which contrast with the two years an year and half needed in USA and in Chile respectively, to reach this size.

Survival was very high (over 90% most of the time dropping to 86% at the end of the study) as shown in Fig 5.

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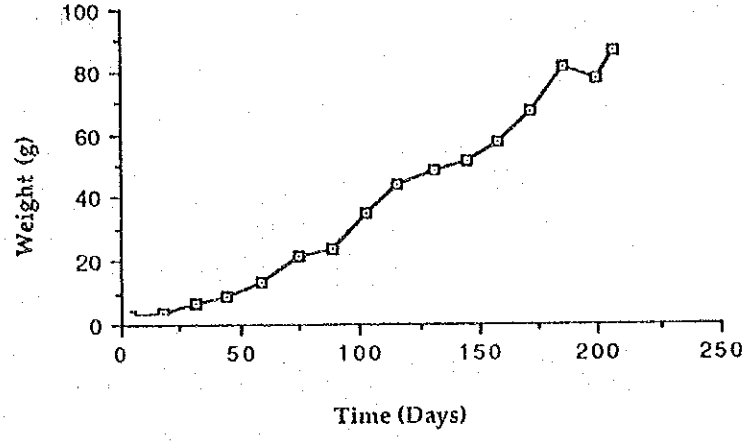


Fig. 4. Growth curve for the Pacific oyster (*Crassostrea gigas*)

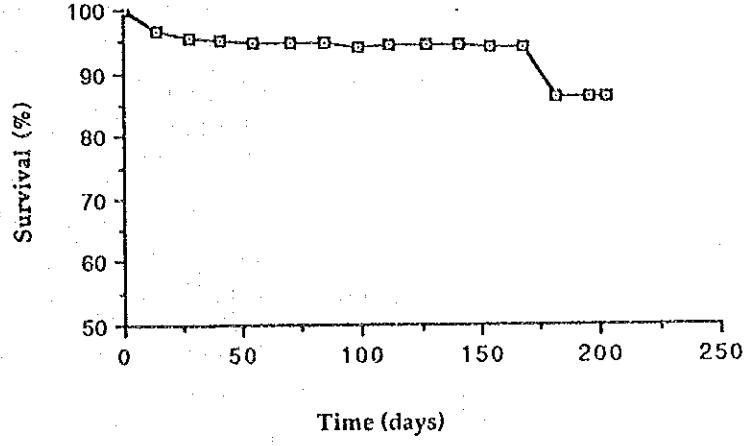


Fig. 5. Pacific oyster(*Crassostrea gigas*) survival throughout the study

Gregarines in different stages of development were found in the shrimp tissue examinated monthly for this purpose. The results of this analysis are shown in Table 1 in which we can notice that the presence of gregarines in shrimp was sporadic and its appearance in the experimental pond was coincident with the appearance in other ponds (with no oysters) at the farm; the latest suggest that the occurrence of gregarines in the pond is not directly related to the oysters and its presence in shrimp may be due to other non determined factors. It is necessary to do more research in order to determine the factors affecting the presence of gregarines.

| MONTH | % GREGARINES (presents in shrimp samples) |
|---|---|
| JUNE | None |
| JULY | 40% Nematopsis or adults (Intestine) |
| AUGUST | 30% Trofozoitos (Intestine) |
| SEPTEMBER | Few Gametocytes (anal vesicle) |
| OCTOBER | Few Gametocytes (anal vesicle) |
| NOVEMBER | None |

Table 1. Occurrence of gregarines in shrimp samples throughout the study.

The results of the microbiological analysis shown the absence of coliforms which means that according to the FDA standards the oysters are suitable to human consumption (Herrera, 1989). Three species of vibrio were detected within the oysters in concentrations considered acceptable, except for the *Vibrio parahaemolyticus* by the standards for shrimp export (there are not standards set up for molluscs) establish by the National Institute of Fisheries. The results are show on Table 2.

| IDENTIFICACION BACTERIANA | CONTEO MAXIMO (ufc/g) | CONTEO MINIMO (ufc/g) |
|---|---|---|
| viables microorganisms count | $2.9 \times 10^5$ | $2.4 \times 10^4$ |
| *Vibrio alginolyticus* | $3.8 \times 10^4$ | $4.4 \times 10^2$ |
| *Vibrio anguillarum* | $1.1 \times 10^3$ | negative |
| *Vibrio parahaemolyticus* | $3.0 \times 10^3$ | negative |
| fecal coliforms | negative | negative |
| fecal totales | negative | negative |

Table 2. Results of the microbiological analysis of oysters .

Phytoplankton population in the pond was composed mainly by diatoms and was maintained by continuous fertilization with urea (a bag of urea was suspended from a pool in the middle of the pond and was replaced by a new bag every time it got empty).

## Conclusions

The oyster rapid growth and high survival observed in this study confirm the possibility to develop mollusc culture in the coastal zone of Ecuador.

Shrimp total survival and growth were good considering that was stocked directly into the pond and was produced using maturation nauplii.

The results obtained demonstrate the possibility of polyculture oyster-shrimp within the same culture pond; this will allow farmers to optimize the use of their shrimp culture facilities and at the same time they will be able to produce other commercially important organism.

Since oysters reached minimum commercial size within five months there is a real possibility to coordinate both cultures (shrimp and oyster) in order to harvest both organisms at the same time.

According to the results obtained in this study, the presence of gregarines in shrimp would not be related with the presence of shrimp in the pond. Since we can't conclude this as a fact, it will be necessary to conduct more research in order to determine the real causes for the occurrence of gregarines within the pond.

In order to determine if it is necessary the installation of a depuration facility for mollusc, further studies stressing the presence of microorganisms in the water an in the molluscs are needed.

It is necessary to realize more polyculture studies using different species of molluscs under different environmental conditions to determine the best suitable species for culture and the areas were the culture can be developed.

Acknowledgments

The authors wish to express their thanks to Mr. Alfredo Fuentes who kindly let us use one pond at his shrimp farm. Our gratitude also to the personal of CENAIM microbiology section for their help during the course of this study.

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Summary

1. Species selection
1) Eighteen references for commercially important species as well as larval identification were collected and reviewed.
2) Surveys on hearsay evidence and fish market observation were carried out over 14 sites.
3) Some 44 species of commercial importance were observed and they were published in a colour pictorial book entitled "A field guide to the edible fishes and shellfishes in coastal waters of Ecuador"
4) Three major candidate species for aquaculture are :
Fam. Centropomidae Centropomus nigrescens Günther, "Robalo"
Sciaenidae Synoscion stolzmanni (Steindachner), "Covina"
Bothidae Paralichthys woolmani Jordan & Wilson, "Lenguado"
[or P. adspersus (Steindachner)]

2. Spawner collection and transportation (Egg collection)
1) The spawners of "Lenguado" and "Robalo" were collected at Ayangue and Salango/Manta, respectively.They were transported to the Laboratory by car taking ca.3 hours.
2) Rearing of the spawners was made in captivity. However, difficulties are: (1) no food ingestion, (2) scale-skin removal (damage),injury and (3) presence of parasites.

3. Feasible utilization of natural fry/juvenile (Seed production)
1) A feasibility study to utilize natural fry is being effectuated twice a month during full and new moon phases at shore waters in front of the CENAIM, since December 1990.
2) There are more fry during the rainy season in which water temperature is higher than the dried season.
3) Mostly occurred fry belong to families Soleidae and Centropomidae including candidate species for aquaculture. A problem for the species identification is remained.
4) During surveys, six kind of fry collecting gear were observed. They were figured and explained to be published.
5) A preliminary experiment on transportation of juvenile "Robalo" by air and car was carried out.It maximumly took seven and an half hours, showing more than 85 % survival rate.

4. Rearing management
1) Rotifer culture fed with T. maculata, I. galvana and bread yeast was made and the best result was obtained with T. maculata.
2) Using natutral fry Achirus sp., rearing experiments were done at the laboratory fed with artemia nauplii, shrimp meat and frozen-dried squid. A heavy mortality was found when metamorphosis occurred.

5. Aquatic environment monitoring
1) During the period from December 1990 toJuly 1992, shore water temperature fluctuated between 22.2 and 33.0 C, whilst salinity varied between 34.9 and 35.6 psu.

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# ACTIVITIES REPORT FOR " FISH CULTURE PROJECT"

The followings are the description of the activities carried out by the Fish Culture Department of the National Aquaculture and Marine Research Center (CENAIM) according to the plan submited to JICA.

## 1 Species selection

### 1.1 Literature review

A review of many books, papers and the basic bibliography concerning culture, reproduction and taxonomy of the native and exotic fish species of commercial interest has been made (Appendix).

### 1.2 Survey on fish market and hearsay evidence

During two years, more than 10 times of fish market visiting/ interview and hearsay evidence have been made at various areas. Then, among 44 species observed, more than five of them were nominated as candidate species for having good perspectives in Ecuador. These species are: "Lenguado" (*Paralichthys woolmani*), "Robalo" (*Centropomus nigrescens*), and "Corvina" (*Cynoscion stolzmanni*), "Cabrilla or Mero" (Genus *Epinephelus*), "Pargo" (Genus *Lutjanua*),. These were regarded as candidates because they show growing big, good marketing price, commonly obtainable, occurrence of the fry according to hearsay evidence etc., and have related species in aquaculture in foreign countries. Especially, among them, "Lenguado", "Corvina" and "Robalo" are highly recommended. The sites visited and/or surveyed for the fry and spawners were Manta, Puerto Cayo, and Crucita; Palmar and Guayaquil; and Tonchigue in Manabi, Guayas and Esmeraldas Provinces, respectively (Fig. 1)

A fish fry and spawner survey based on hearsay evidence was made to know whether fish species potentially available for aquaculture are existing or not, along shore waters. Results obtained are as follows (from North to South):

Esmeraldas (Feb.28 - Mar. 2 ,1991)

According to local people neither fry collectors nor middlemen are presently working for fish fry aside from their shrimp postlarvae (fry) collection. On the other hand "Cabrilla" spawners are available from March to May, though no fry of the species were found in the sea. Regarding "Pargo" and "Lenguado", the fry are found at mangrove areas, whilst "Robalo" are being caught in shrimp ponds.

Manta: (May 13-14, 1991 and 23-25, 1991)

According to Dr. Akamine, "Robalo" is a good species for its aquaculture as the species has a great tolerance to salinity fluctuations and there are many fry in shrimp ponds. On the other hand, it is difficult to capture and transport the spawners alive regardless of the quality and quantity.

Puerto Cayo: (July 23-25 and Sept. 19-20, 1991)

It is possible to capture the spawners of "Robalo" by gill nets during full moon period on October, whilst during winter season by angling. Someone said that it is easy to get adults "Lenguado" and "Robalo" by gill nets. Many "Mero" are captured during full moon period.

San Pablo: (Nov. 29, 1990)

The fishermen found many fish fry (unknown species) but they are usually not alive after collection.

Palmar: (May 9, 1991)

Apart from the shrimp fry collection, people at Palmar have not been gathering fish fry due probably to ignorance of the fish species.

Machala: (Feb. 4-5, 1991)

They have no experience for collecting fish fry. In the past, one of the shrimp fry collectors captured and sold fry of "Jaiba" (portunid crab). However this local people is interested in collecting fish fry for sale if they were

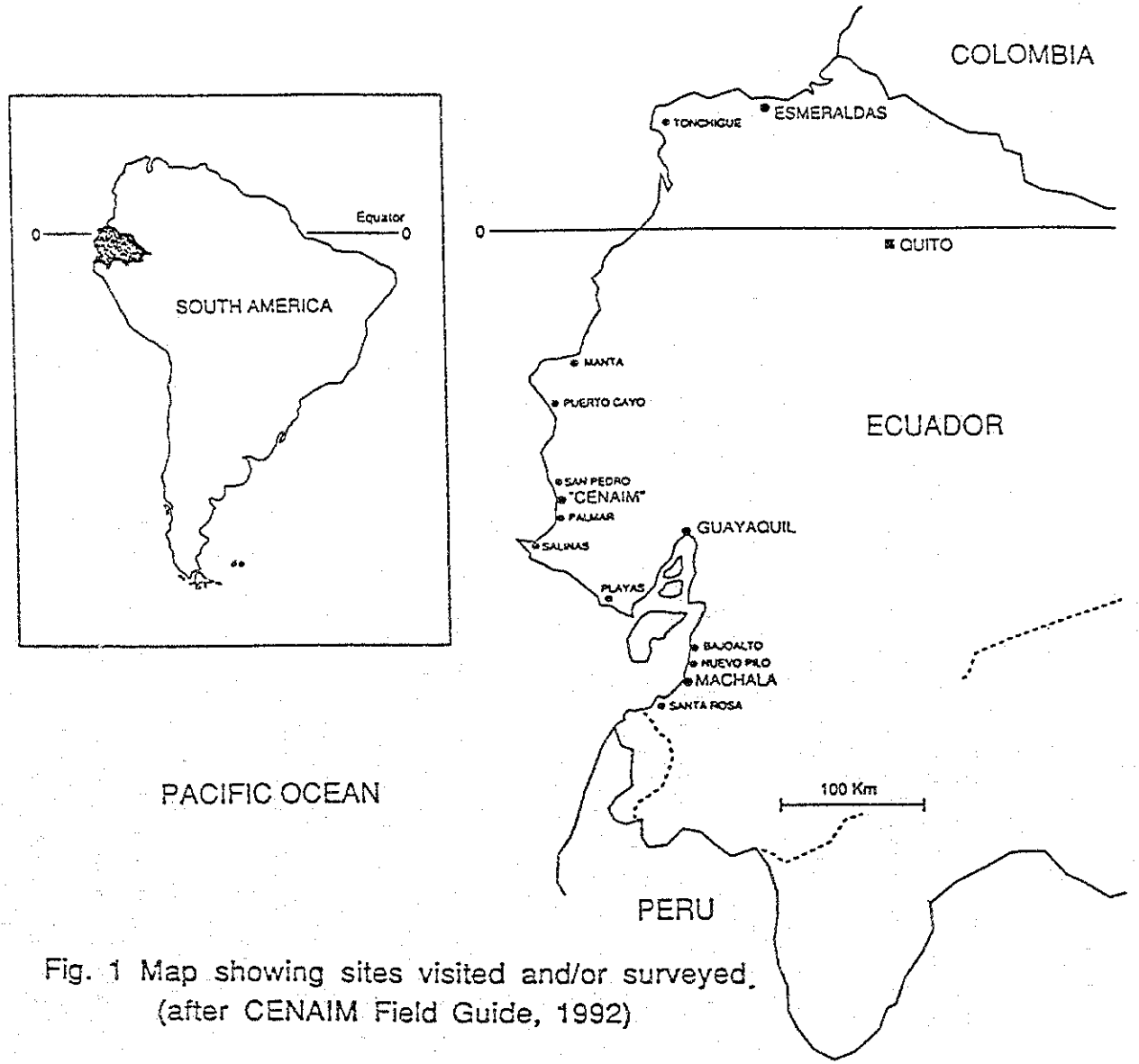


Fig. 1 Map showing sites visited and/or surveyed.
(after CENAIM Field Guide, 1992)

instructed in identification and handling of the animal.

The mentioned species of commercial importance were published in a colour pictorial book entilted "A Field Guide to the Edible Fishes and Shellfishes in Coastal Waters of Ecuador".

### 1.3. Occurrence of wild fish fry along ecuadorian shore waters

At present a large number of shrimp fry are collected by many local fry collectors for its pond cultivation in Ecuador. On the other hand, the wild fish fry are presently not utilized by them, in spite of their good occurrence regardless of the species. Recently there is an enthusiasm for fish culture in this country. Therefore, it is important to know the wild fish occurrence, specially the species of commercially important ones potentially as a candidate for aquaculture. Because those fry might be introduced into the cultivation pond as seedling, aside from natural resources conservation view.

#### Materials and methods

Surveys have been performed during within two days before or after full and new moon phase and during high tide on the day, since December 1990 up to now, as those conditions give more abundant fry according to local shrimp fry collectors. Collection was carried out by a local fry collector who used a triangular net locally called " Tijera". This gear consists of a flattened conical bag made of fine - meshed (ca. 0.9 mm ) synthetic fiber and two pieces of bamboo poles with a wooden or styroform shoe at each end. It had a mouth width of ca. 2.5 m. The survey site was in front of the CENAIM at San Pedro, at wading in waist deep water from 30 to 120 cm, parallel to the shoreline (see Motoh, et al., 1992). One survey consisted of six runs of 150 m distance back and forth taking around 15 minutes each. Heterogeneous fish fry collected were immediately preserved in 5 - 7 % sea water formalin. Later these were sorted out at the CENAIM laboratory. The identification of fish fry was made with the advice of M. Sc. María Herminia Cornejo.

## Results

The survey was carried out twice a month from December 1990 to July 1992 resulting in 40 samplings. During the survey shore water temperature (T) fluctuated between 22.2 and 33.0 °C, while salinity ranged between 34.9 and 35.6 psu (practical salinity scale).

The highest catch was found on January 30, 1991 showing 4,471 fish fry followed with January 20 and April 3, 1992, showing 2,022 and 2,317 fry, respectively. On the other hand, the lowest was found on July 25, 1992, showing 2 fry only followed by August 26, September 9, October 23, November 4, 1991 and July 14, 1992, showing 9, 5, 8, 9 and 9 fry, respectively. These results show that There is more fish fry during the rainy season in which T is higher than the dry season (Figure 2)

Relationships between number of fish fry ("Lenguado" belonging to Families Soleidae and Bothidae) and others are shown in Fig. 2. The higher catch of "lenguado" occurred during the period March - July and November - December.

The results show that the most of groups belong to genus Achirus of Family Soleidae " guarda - boya " followed by genera Eucinostomus and Gerres of Gerridae "mojarras" and Centropomus of Centropomidae " Robalo".

## Discussion

During the present survey it was observed that there were some species of potentially commercial importance such as Paralichthys spp. and Centropomus spp. which have high market prices and are candidates for pond cultivation. However, mostly the problem was the the difficulty to identify genus and/or species. Thus to identify their species, especially those candidates highly recommended. It is also necessary to know annual fluctuations and diurnal occurrences.

All of the local fry collectors were used to throw away the remains of

the collection after sorting out shrimp fry upon dry sand at the beach. This kind of lazy comportment should be forbidden taking into consideration of ecological aspects and conservation of valuable natural resources.

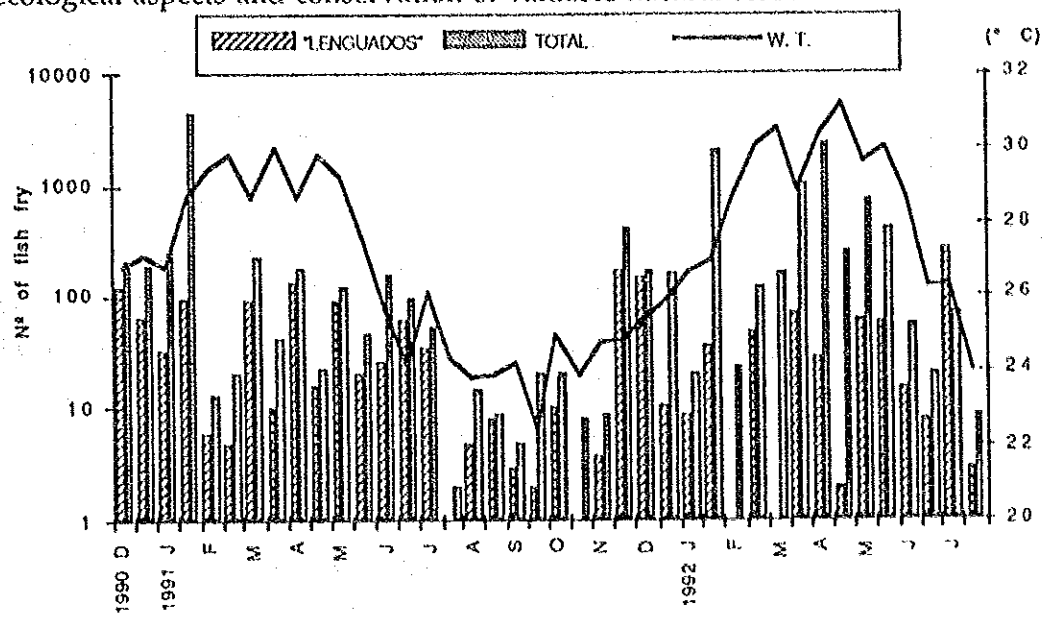


Fig. 3. Relationships between fish fry and water temperature

### 1.3.2 Fry identification

Wild fry samples collected form December 1990 to December 1991 were identified with the advice of M.Sc. María Herminia Cornejo, an expert on ichthyoplankton identification.

During this period, seasonal occurrence of fish fry time distributions could be established after their identification. Ocurrence analysis was divided in two periods; the first period from December 1990 to July 1991 and the second from August to December 1991. Results are shown in Table 1.

Table 1. Occurence of fish fry

| Family | Genus | Percentage % | |
|---|---|---|---|
| | | Period I | Period II |
| Soleidae | *Achirus linectus* | 46.5 | 42.3 |
| Gobidae | *Gobiosoma* sp., *Gobionellus* sp., *Microgobius* sp, *Batigobius* sp. | 0 | 31.0 |
| Centropomidae | *Centropomus* sp. | 22.9 | 15.5 |
| Gerridae | *Gerres* sp., *Eucionostomus* sp. | 25.6 | 4.7 |
| Caranigidae | *Trachinotus* sp. | 0 | 2.4 |
| Others | | 4.6 | 4.1 |

The Gobiidae has been separated from the analysis of the first period, because they are influenced by gust at one season in which we found 2.538 individuals, representing 30%.

Results show that the most of the groups from the first period belong to family Soleidae, genus *Achirus* "Guardaboya" (46.5%) Gerridae, (25.6%) with genera *Eucinostomus* and *Gerres* (latter not completely identified yet) both named "Mojarras", and the family Centropomidae (22.9%) with genus *Centropomus* known as "Robalo".

An occurrence of each group show that the larvae of Soleidae are found in 100% of the samples from the first and second periods, Gerridae 86.7% (first period) and 55.6% (second period), Centropomidae 80.0% (first period) and 44.4% (second period), Engraulidae + Haemulidae 53.3%, Carangidae 45.0% (first period) and 66.7% (second period), Scianidae + Mugilidae + Atherinidae 26.7%, Polynemidae 13.3%, whilst Tetradontidae, Bothidae, Sphyraenidae, Nomeidae and Clupeidae combined 6.7%.

Differences among periods could be explained probably due to physiological changes in relation to reproduction of the broodstock. It is well known that most fish species mate and reproduce during the warm period (December-April).

The larvae appeared mostly during new moon, whilst a few in number appeared during full moon phases in the first period. The opposite occurred during the second period in which the larvae appeared mostly during full

moon. Diurnal occurrence of fish fry in front of CENAIM during 24 hours was also studied. The work was done during mid moon in order to eliminate luminescence influence in fry distribution. The period with the most fry abundance was related with high tide. The larvae which appeared mostly during this observation period belong to the families Soleidae (38.9%), Gogiidae (18.8%), Gerridae (15.6%), Engraulidae (13.0%) and Centopomidae (6.9%).

During May 1991 another fish larvae sampling was done in Porvince Esmeraldas in order to obtain information about fish larvae distribuition in other coastal region. The sites surveyed in Province Esmeraldas were Esmeraldas City, Bajo Alto and Palmar. Results are shown in Table 2.

Table 2. Ocurrence of fish fry at three sites

| Family | Esmeraldas % | Bajo Alto % | Palmar % |
|---|---|---|---|
| Engraulidae | 76.5 | ------ | 77.7 |
| Gobiidae | 23.0 | 25.0 | 23.0 |
| Soleidae | 0.07 | 25.0 | ------ |
| Gerridae | 0.23 | 12.5 | ------ |
| Scinaidae | ------ | 37.5 | ------ |
| Centropomidae | 0.13 | ------ | ------ |

Family Soleidae had low occurrence in this region during the sampling period, in opposite to San Pedro where this family showed the highest occurrence during the same period.

## 2 Egg collection

### 2.1 Spawner collection and transport

During the period from August, 1991 to March, 1992, several surveys on spawner collection and preliminary practice of the transportation have been made for "Lenguado" caught off Ayangue and "Robalo" off Salango and Puerto Lopez. The results are shown in Table 3.

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The problems encountered are no-taking food by the spawners and difficulty to obtain the males.

Table 3. Number of spawners collected or observed.

| No | Date | Place/Gear | Species/Name | Amount | Remarks |
|---|---|---|---|---|---|
| 1 | Aug 27, 91 | Playa Bruja and in front of CENAIM, angling | "Mero" | 0 | It was full moon, but the sky was totally cloudy, too much wind and low temperature. |
| 2 | Aug 29, 91 | Off Ayangue, gill net | "Lenguado" | 9 | Four of the nine were alive. |
| 3 | Sep 3, 91 | Off Ayangue, gill net | "Lenguado" | 8 | Five of eight were alive. |
| 4 | Sep 19, 91 | Off Ayangue, gill net | "Lenguado" | 0 | Trawler's captain interview on board. |
| 5 | Sep 28, 91 | Off Ayangue, gill net | "Lenguado" | 9 | Six of the nine fishes were alive |
| 6 | Oct 8, 91 | Off Ayangue, gill net | "Lenguado" | 8 | Five of eight were alive. |
| 7 | Nov 17, 91 | Off Ayangue, trawler net | "Lenguado" *Hippoglosina tetropthalmus*<br>"Barriga juma"<br>Sciaenidae and "Pampano" | 50-60<br>10-20<br>5-10 | The survey was done by a shrimp boat. There were five surveys at different depths from 2o to 45 m. All the captured fish died during transportation. |
| 8 | Feb 4, 92 | Off Salango, purse net | "Robalo" | 0 | The net was destroyed. |
| 9 | Feb 5, 92 | Off Salango, purse net | "Robalo" | 0 | The sea was very rough. |
| 10 | Feb 6, 92 | Off Salango, purse net | "Robalo" | 0 | The sea was very rough. |
| 11 | Mar. 25,92 | Off Puerto López, puse net | "Robalo" | 1 | The fish had 12 pounds and it was very stresed during the capture and transportation due to its vigorous motility and rough handling by fishermen. |
| 12 | Mar. 26,92 | Off Puerto López, purse net | "Robalo" | 3 | Each with five pounds. |

## 2.2 Spawner handling

At the laboratory of the CENAIM, the spawner rearing has been practiced using 500 l and/or 1 T tanks. The main problem was that the "Lenguado" spawners did not take any kinds of food such as fish and shrimp meat neither live nor pelleted food. This is probably due to the physical damage and stress to fish caused by capturing them with gill nets. The measurements of death spawners are shown in Table 4.

Table 4. Measurements of two kinds of spawners.

| Species | Sex | TL (cm) | BW (gr) | GW (gr) | GSI (%) | Date capt. |
|---|---|---|---|---|---|---|
| *Paralichthys woolmani* "Lenguado" (Caught off Ayanue by gill net) | F | 57.4 | 2.320 | 68.3 | 2.94 | Aug.29 1991 |
| | F | 58.8 | 2.250 | 51.8 | 2.30 | Sept. 3 |
| | F | ca. 61 | 3.180 | 175.7 | 5.53 | Sept. 3 |
| | F | ca. 60 | 2.270 | - | - | Sept. 28 |
| | F | ca. 63 | - | - | - | Sept. 28 |
| | F | ca. 59 | 2.950 | - | - | Sept. 28 |
| | F | ca. 54 | 2.010 | - | - | Sept. 28 |
| | F | ca. 59 | 2.100 | - | - | Sep. 28 |
| | F | 67.0 | 3.660 | 330.7 | 9.01 | Oct. 8 |
| | F | ca. 62 | 2.830 | 105.3 | 3.71 | Oct.8 |
| | F | ca. 64 | 3.340 | 144.1 | 4.31 | Oct. 8 |
| | F | ca. 51 | 2.502 | 260.2 | 10.4 | Oct. 18 |
| | F | ca. 52 | 2.277 | 92.7 | 4.07 | Oct. 18 |
| | M | ca. 50 | 2.234 | 25.1 | 1.12 | Oct. 18 |
| | M | ca. 31 | 535 | 1.3 | 0.24 | Oct. 18 |
| *Centropomus nigrescens* "Robalo" Caugth off Salango | F | ca..86 | 7.961 | Present | - | Mar.25 1992 |
| | - | ca. 60 | 1.633 | - | - | Mar. 26 |
| | - | ca. 61 | 1.701 | - | - | Mar. 26 |
| | F | ca. 62 | 1.814 | - | - | Mar. 26 |

Mortalities of caught lenguados and robalo broodstock caused by many factors already mentioned before, originated to put more effort in catching and transporting juveniles of robalo and lenguado, and to culture them under laboratory conditions until reaching adult and mature stages. Places