TITLE XII PROJECT TO ASSIST IN THE DEVELOPMENT OF IN-COUNTRY PRODUCTION OF BRINE SHRIMP (ARTEMIA) FOR USE AS FOOD FOR AQUACULTURE ORGANISMS IN INDONESIA

Dr. Paul D. Maugle
Department of Food Science and Nutrition
University of Rhode Island
Kingston, RI 02881
U.S.A.

ICMRD

INTERNATIONAL CENTER FOR MARINE RESOURCE DEVELOPMENT
126 WOODWARD HALL UNIVERSITY OF RHODE ISLAND
KINGSTON, RI 02881 USA

July 15 - August 6, 1984

CONTENTS

SCOPE OF THE PROBLEM

There is a serious shortage of Artemia cysts in Indonesia. This is a primary contributing factor which limits overall hatchery production of Post-Larval (PL) shrimp to approximately 10 percent of capacity. A list of Penaeid Hatcheries, their locations and approximate production capacity (581 million PL total capacity) is attached in Appendix I. The low hatchery production of Post-Larvae has resulted in widespread understocking of Indonesia's 180,000 Ha of brackish water ponds. Where traditional farming techniques are not utilized, stocking rates ranging from 5,000 to 10,000 PL/Ha/season are employed. Thus, shrimp ponds often produce between 150 to 300 kg/Ha/six-month growing season.

Indonesia has embarked on a National Shrimp Development Program with a mandate to bring the production of Post Larvae to approximately 1.5 billion over the next 5 years. In accomplishing this goal, the Government of Indonesia (G.O.I.) plans to construct 266 hatcheries.

PROJECTED HATCHERY CONSTRUCTION

YEAR	1984	1985	1986	1987	1988	5-YEAR TOTAL
LARGE-SCALE HATCHERIES	65	+1	+3	+1	+1	71
SMALL-SCALE HATCHERIES	59	+32	+29	+36	+39	195
						266

The Department of Fisheries/Jakarta projects that a large-scale hatchery would produce 40,000,000 Post Larvae (PLs)/year, and a small-scale hatchery would produce 4,000,000 PLs/year. Present larval feeding practices in Indonesia's hatcheries are schematically outlined in

Appendix II and include the use of approximately 25 brine shrimp Nauplii (BSN) per Post larva (PL)/day. Considering that the feeding of Artemia begins at the Post-Larvae stage of development and is continued until they reach a 20 day old animal (PL20) and is only done once a day, it is not difficult to understand why only 10 percent of the shrimp survive to be stocked in ponds. In typical Western Hemisphere hatcheries, feeding begins at Zoea/Mysis stage until PL 7-10 day or approximately 20 days.

BRINE SHRIMP UTILIZATION

ASSUMPTIONS:

MACROBRACHIUM: 20 PLs/Liter/Production
4000 BSN/L/day feeding level
20-30,000 BSN/gm Great Wall cysts
45 day larval cycle

To produce 1,000,000 Post Larvae Fresh Water Prawn, 200 million BSN would be required per day. Great Wall Artemia yield, on the average, 20,000 BSN/gm. Thus, a 45-day cycle requires 450 kg of Great Wall cysts. Bio-Marine or San Francisco brand Artemia, which yield approximately 100,000 BSN/gm, would require 90 kg of cysts per cycle. Brazil Artemia cysts yield 230-240,000 BSN/gm.

PENAEIDS: 5 Post larvae/Liter production.
25 BSN/PL/day.
20-30,000 BSN/gm Great Wall cysts

To produce 1,000,000 PL 20 Penaeid shrimp larvae would require 25 million BSN per day. In Indonesia, feeding with brine shrimp begins at post larvae and continues for 20 days. In typical Western Hemisphere hatcheries, feeding begins at Zoea/Mysis until PL 7-10 or approximately 20 days, and feeding levels are greater than 25 BSN/L/day.

Thus, 1,000,000 Post Larvae using Great Wall Artemia would require 25 kg during the 20 day feeding cycle and San Francisco or Bio-Marine cysts would require 5 kg/cycle.

These are only estimates. Actual usage figures in the case of Penaeid Hatcheries is much less and ranges from 10-12 kg/month of Great Wall Artemia cysts. Hatching rates as high as 17% have been recorded with Great Wall brand cysts placing BSN yields at approximately 30,000/gm of cysts.

ESTIMATE OF QUANTITY USED

Mr. A. Kahar Rasul, Director of Brackishwater Pond Production in Central Java, presented a concise example of typical hatchery feeding practices. Among the seven hatcheries located in Central Java, PL production ranges between 600,-800,000 PL15/6-month season at a survival rate of 40 to 50 percent. It was his estimate that each hatchery utilized approximately twenty-two 500g cans of Artemia cysts/month.

Twenty-two cans of Artemia x 500g = 11 kg/month x 6 months = 66 kg/ 6 month operations in a large-scale hatchery. This would place the present requirement for Artemia in the 37 existing Penaeid hatcheries, if all were brought up to total capacity of 581,764,000 PL/year at 14.5 MT.

This analysis does not include the use of Artemia in freshwater prawn hatcheries. It is estimated that the larvae require 50-150 BSN/ day, depending on the life stage. Each hatchery could require from 250g to 1 kg Artemia/500,000 freshwater prawn/day throughout the 30-40 day larval cycle depending on temperature. In a worst case situation, 1 kg/ day x 40 days x 6 months = 240 kg/6 months operating in prawn hatcheries. Presently, three out of the 5 prawn hatcheries are working at this capacity, thus perhaps 1 MT of cysts are utilized annually among these three hatcheries.

DANGER OF IMPORT DEPENDENCE

Artemia cysts are available but at a cost of between 35-70,000 Rp/16 oz. can; thus, at prices greater than 100 USD per kilogram, hatchery management strategy is extremely limited. A semblance of production can be maintained in hatcheries if Artemia are fed to the shrimp and prawns at low levels and only for a limited time period. Another problem is the use of cysts with low hatching rates, high levels of contaminants or poor nutritional value. These qualitative problems, although not obvious, often result in variable production and unexplained massive mortalities of shrimp larvae.

Indonesia's predicament, when taken in view of the world Artemia market situation, rapidly and progressively becomes worse. Initial shortages of Artemia cysts developed during the mid-1970's due to increased demand by aquaculturists. In the late 1970's new supplies, including those resulting from inoculation of ponds in developing countries, came on the market temporarily alleviating the situation. Presently the growth of the worldwide aquaculture industry and the aquaculturists' awareness of nutritional quality has caused the price of high-quality Artemia cysts to rise to approximately $50 USD/kg. The demands of an expanding aquaculture industry in developed countries have made Artemia cysts available to aquaculturists in developing countries only at a premium price, and then, as I have observed in Indonesia, the majority of the cysts available are of poor quality.

Should Indonesia wish to increase production at the 37 Penaeid hatcheries and 3 Macrobrachium hatcheries now in operation, and assuming expansion with the 5 additional large-scale penaeid hatcheries that the Asian Development Bank is proposing, it is projected that approximately 20-25 metric tons of Artemia cysts would be required on an annual basis. This assumes the use of Artemia in hatcheries as outlined in Appendix II.

Proper coupling of in-country Artemia production to hatchery usage is necessary in Indonesia for successful aquaculture development. With an annual requirement of 20 metric tons to properly operate the existing and future hatcheries, can the G.O.I. afford to pay $50/kg on the international market? If so, is this a realistic long-term solution? An

alternative solution to the problem of cyst availability is the coupling of cyst production to Indonesia's requirements. With the added potential for product development and export market, Artemia cyst production is an especially attractive solution to Indonesia's aquaculture needs. Solar salt ponds are already operated on a large scale throughout Java and Madura Islands.

During the dry season 50,000 Ha of Indonesia's 180,000 Ha brackish water ponds are altered into salt tables or drying platforms. If only 5 percent of this area were converted to Artemia production as much as 100 metric tons of Artemia cysts could be produced. This estimate is based on published production estimates from Thailand of 40 kg/Ha/dry season. In practice actual production would depend on the farmers' capability, availability of low cost organic fertilizer and cyst processing facilities.

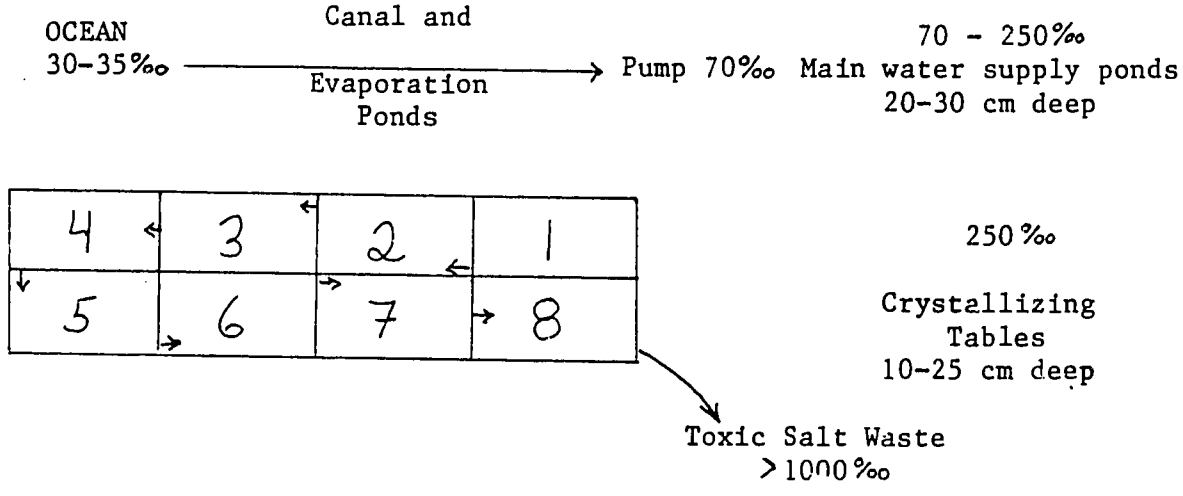
LARGE SCALE DEVELOPMENT

In Madura and East Central Java, the State Salt Enterprise controls 5,750 Hectare of salt production facilities. See map of Madura Island in Appendix III. The facilities are distributed as follows:

Location	Total Area Ha	Brine Storage Area
Sumenep Madura	1,470	-
Nenbakor Madura	1,180	-
Pamekasan Madura	980	1 pond, 10 Ha

Location	Total Area Ha	Brine Storage Area
Sampang Madura	1,150	2 ponds, 18 + 16 Ha
Gresik Manyar, Java	415	-
Gresik Puhlt, Java	555	-
Total Area	5,750 Ha	44 Ha

Mr. Momo Ratmawidjaja, Technical Director of Production, schematically described the salt drying process presently in use, below.



The government-owned salt ponds on Madura Island and N.E. of Surabaya are suitable for integrated Artemia - salt production. Advantages for locating a long-term, large-scale operation in this area include 1) demonstrated technical and managerial skills on the part of the State Salt farm staff members, 2) established infrastructure geared to handle large quantities of material, 3) a large and skillful labor force, 4) 200 years of operation in some areas, 5) 5-6 month dry season, and 6) impermeable soil. The layout of the salt farms on Madura Island (as seen in Appendix III) could, with minor modification, allow the integration of Artemia production without disturbing salt production.

The evaporation ponds are estimated at 6/7 of the total area; 6/7 x 5750 = 4929 Hectares of potential Artemia culture area. At the Sumenup Salt Farm the evaporation ponds had an average depth of between 20-30 cm. When originally constructed they were 45-85 cm in depth but have become silted-in during the long years of operation. With either dragline or a dredging operation an adequate number of these ponds could be put into suitable condition. Based on production estimates of 40 kg/Ha/dry season in Thailand, it is estimated that 750 Ha would be required to produce 30 metric tons of Artemia cysts per year.

In further discussions with Mr. Momo Ratmawidjaja, he expressed enthusiastic interest in demonstrating the potential of Artemia production in ponds within the salt industry complexes. As Artemia required deeper waters (50-80 cm range) to keep temperature within optimal growing limits, he recommended we utilize the brine storage ponds which have recently been constructed in Palmekasan (10 Ha) and Sampang (34 Ha) areas. These ponds were constructed for high saline water storage ($>100‰$ but $<180‰$) during the rainy season, which allows the farms to accelerate the salt drying process at the beginning of the dry season. It is estimated that during the rainy season as much as 1,700 kg of Artemia cysts could be produced in these ponds, if properly managed. Diagrams of these salt farms are presented in Appendix III.

Upon my suggestion that a collaborative effort be established between the Directorate General of Fisheries and the State Salt Industry, it was further suggested that the infrastructure and fiscal arrangements be handled by the salt industry and technical personnel be

provided by DGF and perhaps USAID. I would recommend that a separate grant/loan be provided for initial one-year operating expenses with further amplification when larger numbers of salt ponds are required.

Mr. Burnhanuddin Lubis, Director of Production, D.G.F., during the last week of July 1984, has upon Mr. Momo Ratmawidjaja's suggestion initiated contact with:

Ir. Soesanto Sahandjo
Director of Programme Development
Ministry of Industry
Director General for Multifarious
Industries
Jl. Gatot Subroto
KAV 52-53, 14th Floor
Jakarta, Selatan
Ph. 514038

It is further recommended that arrangements for initial use of the deep brine ponds with expansion into a large scale production system in the Madura area should be followed up by USAID/DGF. This could be done on a collaborative basis with salt industry representatives providing infrastructure and perhaps some of the operating expenses.

Mr. Momo Ratmawidjaja was particularly interested to learn how brine shrimp could enhance salt production. Evaporation ponds in other areas outside of Indonesia which do not contain Artemia are dominated by

benthic blue-green algae, which produce a mucous material slowing evaporation. When Artemia are present and the ponds receive organic fertilizer, the blue-greens are out competed by other kinds of algae that Artemia feed upon; thus, there is no mucous production. The feces and soluble excretory products from Artemia are carried into the crystallization ponds and are used by bacteria (Halobacterium) covering the pond bottom. The deep-red color of these bacteria causes solar heat to be retained in the ponds, enhancing the evaporation process. Biologically-managed solar salt ponds operate at a higher degree of efficiency and temperature and provide Artemia cysts as a by-product. Mr. Momo was particularly interested in the fact that Artemia pond culture would reduce the magnesium and fluoride in the pond, thus yielding a higher quality salt product as well as a secondary product, Artemia cysts, for sale to in-country hatcheries.

A further by-product is that periodically the adult Artemia would have to be harvested in order to maintain population densities. These animals contain high hormone levels, undergo 2-3 week reproductive cycle, and have been utilized to feed broodstock Penaeus japonicus in Brazil with success. Trials might also be held to evaluate Artemia as a feed to enhance milkfish and Penaeus monodon reproductive development.

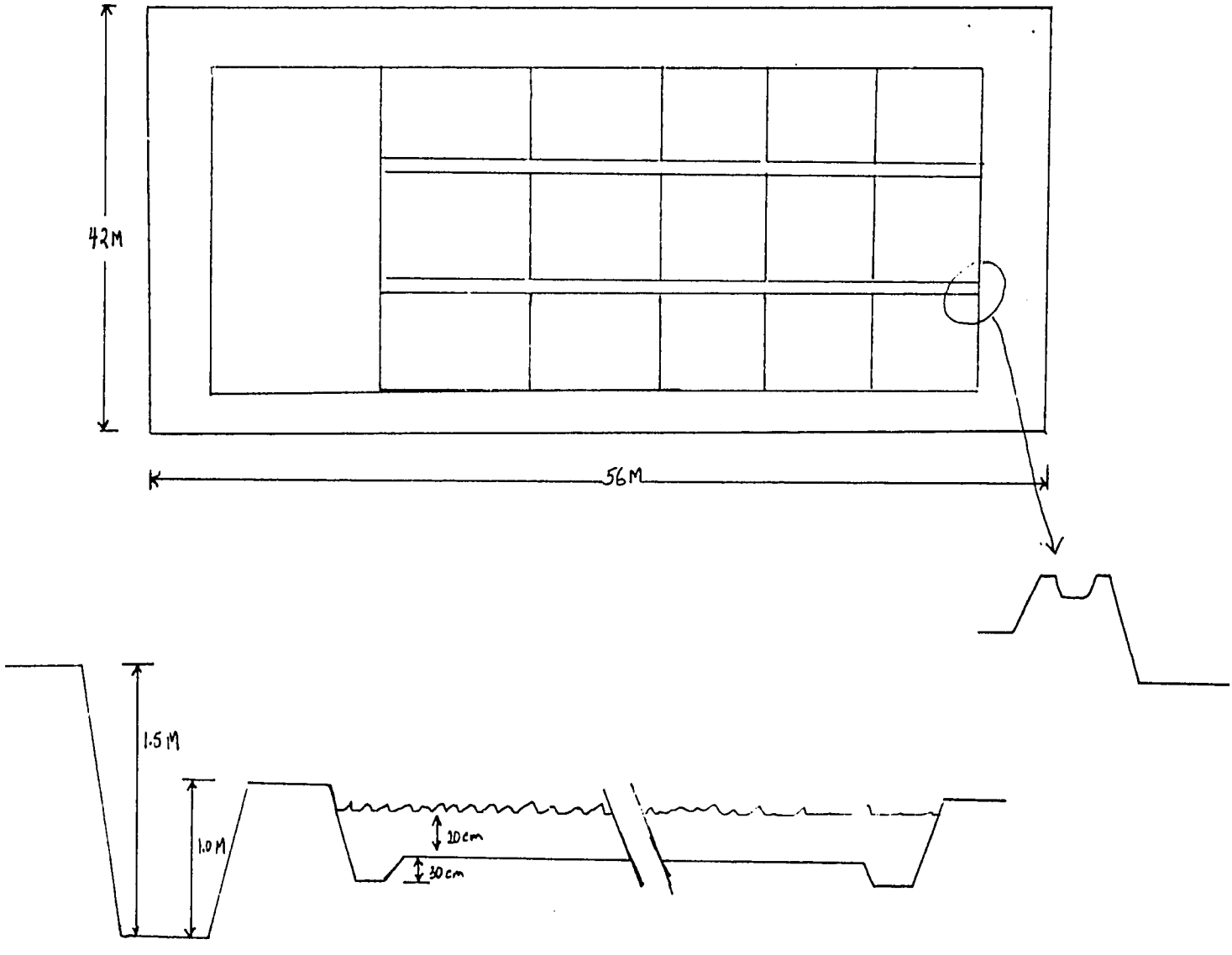
SMALL SCALE DEVELOPMENT

Two Artemia demonstration ponds were reviewed during site visits. The first in Gondon, Bali, is under the control of the Mr. Alipromo, Director of the Department of Inland Fisheries, and the second located

in Sampang, Madura and is controlled by Mr. Soewito, Director of the Department of Natural Resources, with infrastructural support provided by the Production Department, Mr. Lubos, Director.

GONDOL - BALI

The Gondol, Bali site included 15 small ponds, approximately 200 m^2 each ponds, with an attached small-scale evaporation unit.



The ponds require deepening and are built on coraline and coral/ sandy soil which causes extensive seepage. They are also 20-50 cm in depth which will lead to temperature problems during culture operations. More important, however, is the seepage problem. In order to maintain optimum temperatures, the depth of pond will have to be maintained at a minimum of 50 cm depth. Increased seepage will cause an increase in water temperature. In order to lower the temperature the pond manager will have to pump in more water which will reduce the salinity. At salinities lower than 90‰ Artemia predominantly produce napulii, and at salinities lower than 60‰ predator species will feed on the Artemia.

In order for this demonstration pond system to begin operations, the following modifications should be put in place.

1. Deepen the ponds to 50-80 cms.
2. A water distribution system including a small pump should be put in place.
3. Seepage is a problem, as this site has a coral rock base and will interfere with the success of this project. Thus, Bentonite or drillers mud available from many sources throughout Bali should be applied to this pond system before any evaluation trials begin.

The management of this pond system should include a well developed experimental design. Statistical analysis of the results will greatly assist in the assessment of future Artemia development in Bali, and throughout Indonesia. It is imperative that the experimental design test only levels of fertilization during the first dry season. Evaluation of local feed by-products (rice brand etc.) and suitability of

various strains should wait until the next season. To do otherwise would confuse the results and would not allow the production of sufficient Artemia cysts for quality evaluation. It is also important that the strain selected for inoculation in these initial trials be previously tested for nutritional suitability, for use in culture operations.

Research conducted by the University of Rhode Island has shown that the nutritional quality of Artemia cysts depends on the pond environment in which they were produced. The brine shrimp transfer not only nutrients but also contaminants such as pesticides and heavy metals to cultured fish and shrimp. The quality of the brine shrimp can be predicted on the basis of chemical analysis. The chemical analysis of cysts produced under controlled environmental and nutritional conditions can confirm the appropriateness of pond management techniques. Proper processing of the cysts will yield good hatchability.

Thus, it is important that samples of the inoculating strain(s) be saved for nutritional and chemical evaluation if not already available. The fatty acid profile or ratio is indicative of nutritional quality. Low levels of 20:5W3 indicate poor nutritional quality for marine animals.

SAMPANG - MADURA

The Sampang, Madura demonstration pond site is 1.18 Ha and is subdivided into 9 smaller ponds. These ponds are of irregular shape. See drawing in Appendix III. There are three ponds of similar shape and

size, but they vary in depth from 40-80 cms.

In discussions with field representatives at the Jepara Station, it was indicated that various Artemia strains, quantity of fertilizer and quantity and type of feed by-products would be evaluated. Again, as recommended above, a strict experimental design has to be set out, and all who have a hand in the operation should adhere to it without deviation.

Salt industry representatives in Surabaya indicated they were watching how this demonstration pond was developing and could be favorably influenced to become involved with Artemia production if the results were positive.

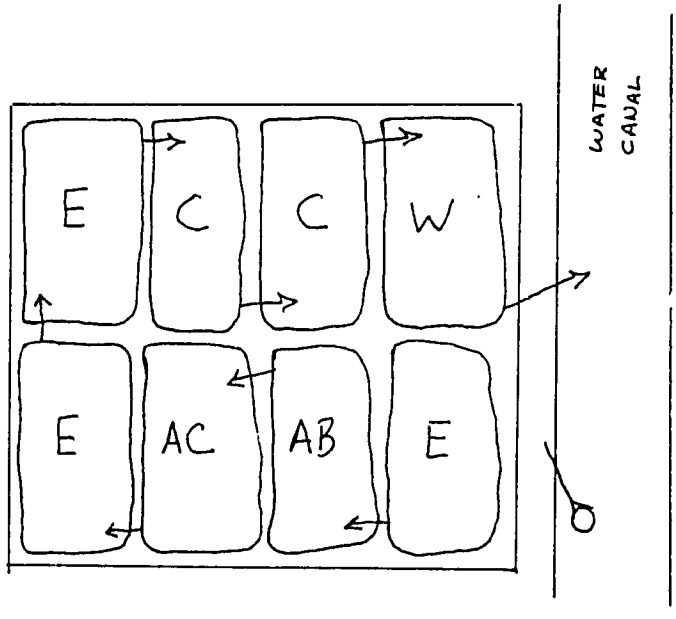
The demonstration ponds at Sampang should be made to a uniform depth. This will remove a temperature variable and clarify the evaluation of any experimental results. Experiments to test the effect of the temperature/depth interaction on growth, biomass and cyst production could still be evaluated by varying the water depth, yet the culturist would retain the management option to add water, reducing salinity and/or temperature to optimum levels.

Any support from USAID to this project should only take place if an experimental design showing clear evaluation of results is adhered to. This should be included in any agreements with D.G.F. supervised projects.

PATI - REMBANG

I would suggest that a small-scale demonstration project be developed by the D.G.F. in the PATI, Central Java area, under the direction of Mr. A. Kahar Rasul, Director of Brackish water production. An important factor in situating a demonstration project in PATI area is the interest and curiosity of key farmers. Their influence in spreading new ideas on pond management techniques (feeds, fertilizer, etc.) has made this area one of the most productive. The ponds in this area are better suited for Artemia production, as the dykes bordering the salt tables are higher and the modification of these ponds would be less costly. Small-scale farmers in this area work a total of 1,400 Ha of Tanbak, of which 800 Ha are modified annually for operation as salt drying ponds during the dry season. On a seasonal basis the average production from ponds in the PATI area includes: 100-150 kg shrimp, 300 kg/Ha of milkfish and 40 tons of salt.

Demonstration ponds in this area could be built to include 2-4 large 1 Hectare Artemia production ponds within the scheme of salt evaporation units.



AB = Artemia Biomass

AC = Artemia cyst production

E = Evaporation

C = Crystallization

W = Waste

BARRIERS TO SUCCESS IN SMALL-SCALE ARTEMIA/SALT FARMS

A: SAMPANG, MADURA

1. Wide-scale conversion to deeper ponds would have to take place before Artemia production could begin.
2. Loans to salt producers would have to be paid before any Artemia could be produced.
3. Each district would require a demonstration pond which would serve as a training center.
4. Marketing of small amounts of cysts will be problematic, with large losses occurring due to poor handling or processing.
5. Marketing of small farmers product in the Madura area is tied to the successful development of a large-scale market infrastructure by the state salt enterprise.

B. GONDOL, BALI

1. Numerous physical limitations need to be overcome before this project can begin.

    A. The size of ponds at the demonstration ponds in Gondol is very small and quantity of cysts produced may not be sufficient to allow biochemical/nutrition evaluation.

2. Area on N. Coast Bali does not appear suitable for future expansion into larger units. Any future development should be

developed at the south shore small-scale salt farm. However, this area may have problems with pollution in the future.

IN HATCHERY PRODUCTION OF ARTEMIA NAPULII

The technology for this system, initially developed at the Artemia Reference Center (ARC) in Belgium, was transplanted to Indonesia by Dr. McVey and members of the ARC. All systems were operated on small scales, at all of the three locations observed or reported, Pasar Mingo, Jepara or Prigi. Mr. Mulyons Sastaowidjaja, Deputy Director of the Brackishwater Aquaculture Project in Surabaya, has provided some preliminary data in his attached report on the optimization of this system at the Prigi Macrobrachium hatchery. From this small scale (0.5 ton) recirculating system, Appendix IV, approximately 600-800,000 B.S.N. were produced per day.

The Jepara system is a low energy input, static tank culture system which is claimed to produce 300,000 BSN/day. Both systems utilize an Italian parthenogenetic strain and feed rice brand. The Prigi system also feeds yeast. Both groups were interested in improving the fecundity but additional feed only caused pollution. They were very interested in testing fish liver oil (at a 4-6% level) in combination with either wheat flour or powdered rice bran.

The differences in production directly correlate to the amount of energy and interest on the part of the aquaculturist. In order for sufficient nauplii to be produced to meet the requirements of a

hatchery, a large-scale and/or intensive culture effort will have to be mounted.

In discussions with Mr. Howard Deese, USAID/Yojakarta, the recommended daily hatchery requirement for Artemia in a Macrobrachium hatchery was estimated at 4,000 BSN/Liter/day. Post-Larvae prawn production was estimated at 10-20 PLs/Liter with 100,000 BSN/gm of cysts available for feeding when Bio-Marine or San Francisco brine shrimp were used.

It must be stated that the small-scale fisheries project does not use Great Wall Artemia cysts, as do all the other hatcheries I have visited. This brand of Artemia cyst was avoided because only 20-30,000 BSN are obtained per gram of cysts on a dry weight basis.

Artemia purchases are a large part of every operating budget, but in-hatchery production of BSN would, if scaled appropriately, alleviate only part of the problem. In Indonesia most penaeid hatcheries are feeding Artemia beginning at Post-Larvae stage, resulting in production far below actual capability. The in-hatchery production of Artemia and rotifers and shrimp feeding practices should be considered when planning future technical assistance. Practical training in live food production and feeding practices should be an integral part of USAID's future assistance package.

Realistic expectations of in-hatchery brine shrimp production are estimated at 250,000 BSN/day/0.5 ton tank. This could partially supply

the Artemia requirements in some small-scale hatcheries. Large-scale tank production of BSN has not been demonstrated in Indonesia, and direct extrapolation of potential production figures should not be employed in future planning. In the future, the utilization of "spare" hatchery tanks which in many cases are in the range of 10+ tons capacity may be possible, but to date, no examples of demonstrated technical ability to do so exists in Java. Moreover, the fiscal capacity to manage large-scale projects, dealing with many hatcheries at the same time, will present more infrastructural than technical problems.

SITE VISIT TO POTENTIAL ARTEMIA AND PENAEID SHRIMP CULTURE SITES IN N.W. JAVA WITH HUSNI ASSOCIATES

BACKGROUND: A major limiting factor to productive use of shrimp hatcheries in Indonesia is an inadequate supply of Artemia live feed. Imported from various parts of the world, at prices ranging from 35-70,000 RP/500g can, in very limited quantities, the Artemia cysts (eggs) are not used on a daily basis in the hatcheries. As a result, post-larvae (seed stock shrimp) survive poorly and grow slowly in ponds and hatcheries throughout Indonesia.

Indonesia has begun a National Shrimp Development Program with a mandate to bring production of post larvae to approximately 1.5 billion over the next five years. To accomplish this the G.O.I. plans to construct 266 hatcheries.

PROJECTED HATCHERY CONSTRUCTION SCHEDULE

YEAR	1984	1985	1986	1987	1988	5-YEAR TOTAL
LARGE-SCALE HATCHERIES	65	+1	+3	+1	+1	71
SMALL-SCALE HATCHERIES	59	+32	+29	+36	+39	195
						266

To produce 1.5 billion post-larvae would require 37.5 tons of Artemia cysts annually. The Department of Fisheries has begun a small-scale (1 Ha) demonstration pond project in Madura but results are not yet available.

On August 1, Mr. Soebekti, Mr. Raymond Gan, members of the Husni & Associates Resource Development Consultants, and I traveled to two sites in N.W. Java to look at site potential for shrimp and/or Artemia culture. The first site located adjacent to the Village of Ciparage consisted of approximately 200 Ha of tambak.

There is a potential for construction of up to 350 Ha of tambak. The site is located 23 km north of Karawan, along a dirt and stone road which in its present state may become impassible during the rainy season.

There were approximately 40 Ha of salt drying ponds that appeared not to have been operated for the past 3-4 years, and were now being converted to shrimp growing ponds. Water quality data was taken from a pond approximately 150 M from the ocean. Data included 25 salinity, 30°C pond temperature, pH 6.9-7.0 at 20 cm depth. Barometric pressure of 30.02 and wind velocity of 20-22 km/Hr. Salinity was also taken at a bridge over a canal connecting the river to the ponds, approximately 0.5 km from the coast at high tide and was found to be 0‰.

In discussions with local farmers, we learned that only 3+ months of high salinity water existed (Sept.-Nov.+) and that during the rainy season the tambak area flooded with fresh water from a nearby inland lake.

It is recommended that large-scale investment at this site by this private group be avoided. The village needs a road. Tambak improvement

to attract government funds from a possible national aquaculture program could be possible. However, the site is situated in a rice growing district which puts the aquaculturist at the mercy of whatever the rice growers put into their paddy. Pesticide and insecticide pollution will limit any tambak production from this area.

In visiting the local fish market, only Penaeus merginsis, not P. monodom, were observed. Perhaps this is due to fishing technique, but I believe that P. monodom, the higher-priced shrimp, is not abundantly distributed in this area.

The second site visited by the group consisted of four salt drying areas west and east of Eretan between the highway and the ocean. The salt drying units were 3-4 Ha in size, surrounded by a large berm 1-2 M high, with the evaporation and crystallization units located inside.

The soil in this area consisted of silty sand and clay mixture at both the western and eastern sites along the road. In discussions with local people and the village chief, Mr. Tamsa, the vertical structure of the soil consisted of ½ M of sandy clay at the surface, then 3 M of clay (red or black), and finally 3 M of gravel. He mentioned that ponds constructed with red clay did not produce algae and had a slow evaporation rate.

At the east Eretan site approximately 600 people were involved in salt production during the dry season, but became rice paddy farmers during the rainy season.

Quality problems began shortly after the farmers started producing salt in 1960. The salt made during poor evaporation conditions produce caustic soda and not sodium chloride. The change in weather conditions, high levels of silt in the salt water and expansion into larger number of ponds with poor soil types which have low productivity resulted in slow evaporation. The farmers did not try to grow green algae by fertilizing but rather added $CaCO_3$ to the crystallization units to enhance precipitation. The resulting product was 50% caustic soda.

Since 1960, when salt production began in this area, production ranged from 60-80 ton/Ha and sales to Jakarta brought prices approaching 50 Rp/kg. Recent quality problems and political/economic pressure from the State Salt Enterprise has limited access to the Jakarta market. The farmers are interested in doing something different but have no plan for future activities during the dry season.

I recommend that sites in the Eretan area be strictly evaluated using the site selection criteria attached. Existing hatcheries with Artemia growing potential should be evaluated as to their availability for sale to the commercial sector.

Although the economic environment for commercial Artemia production appears good in the Eretan area (i.e., 200 Ha @ 20-40 kg/Ha producing 4-8 tons/dry season potential), there are a number of built-in disadvantages.

1) Close proximity to paddy rice cultivation area.

2) High silt levels in water from sea.

3) Rapid erosion has decreased workability of ponds.

4) The salt sample needs to be evaluated to see if Artemia will live and reproduce in it.

5) Soil quality is variable, containing H_2S and/or iron, both can be toxic to Artemia.

Follow-Up Actions

1) Hatch out Artemia in 30-35 salinity using salt sample. Feed them 2 times daily with wheat or rice flour. After 3-4 weeks, raise salinity to 110 using salt sample from Eretan. After 1-2 weeks they should produce cysts which can be found along the side of tank above water line.

2) Analytically examine the salt sample for the following criteria: a minimum of 94.7% NaCl; less than 40 ppm sodium sobagai $NaIO_3$; less than 100 ppm Fe_2O_3; less than 1% Kalim dan Magnesium sebagai Ca; less than 2% SO_4 and find out how much Pb, Hg, Cu Al are contained in the salt.

Should both these criteria be successful, then the site could be evaluated for physical/chemical suitability of the soil and water and finally economic arrangement could be made.

SITE VISIT ITINERARY

15 July - Arrive Jakarta

16 July - U.S. Embassy: Met with Mr. Kevin Kushing,
Mr. Howard Deese, Mr. Sa'ud Mohamad

- Directorate General of Fisheries: Mr. Buehoduddin Lubis
(Director of Production Development)

- Pasar Minggu, Inland Fishery Research Station,
Mrs. Haniah Saharto, Director

17 July - Travel to Bali

18 July - Dinas Perikanhn Propinsi-Bali: Mr. Gede Harmony
Director, Mr. Wisnawa Assistant

- Travel by car to Gondol. on N. Coast.
met with Mr. Wadoyo, S.B.P.D. Gondol, and
Mr. W. E. Vanstone, RMI milkfish specialist

- Visited Artemia Demonstration Pond

19 July - Travel by car to Penaeus monodom Hatchery
"Windu mas", Sukawati - Gianyar.
Mr. Halim Tansil Director, Mr. Budi Lamb Shrimp Culturist

20 July - Travel by car to S. coast salt drying cooperatives

- Travel by plane to Surabaya, Java.
Met Dr. J. Wiryanti, D.G.F./Jakarta, and
Mr. Sa'ud Mohomed USAID.

21 July - Dinas Perikanan Duerah Propinsi Paerah Tingkat 1 Jawa Timur Met with Mr. Djoko Tribawono, and Mr. Mulyono Sastrowidjojo

- Traveled by car and ferry to Madura with Dr. Wiryanti
- In Sampang met with Mrs. Imam Syakri, wife of the director. Visited the Demonstration Ponds.

22 July - Traveled from Palmekasan to Sampang: met Mr. Syakri discussed Demonstration Pond plans.

- Traveled by car from Palmekasan to Sampang to Bangkalan along the North Coast of Madura to Sumenep.

23 July - At the Sumenep, D.G.F. fisheries office: met with Mr. Mochtar, Lawyer and Chief of Economic Department of the Local District Authority

- Traveled to the State Enterprise of Salt Production, a 1,470 HA salt production facility: met Mr. Suroso assistant director, and Mr. Mohamad Ending, Project Coordinator.
- Returned to Surabaya from Sumenep along S. Coast via Pamekasan, Sampang and Kamal to Surabaya via ferry from Madura Island

24 July - Surabaya Department of Fisheries met with Mr. Djoko Tribawono and Mr. Mulyono Sastrowidjojo

- Surabaya: Ministry of Industry, met with Mr. Kukuh

24 July - Surabaya: State Enterprise of Salt met with Mr. Momo Ratmawidjaja, Technical Director of Salt Production.

- Travel by plane to Semarang

25 July - Dinas Perikanan Prop. Jateng: met with Mr. A. Kahar Rusul, Brackishwater Aquaculture Development Director

- Traveled to Jepara: met with Mr. Muhammad Murdjoin who will work in Sampang Demonstration Pond and Mr. Sudjiharno Sainum Pond Production Manager of Jepara Station. Met Mr. Lubus Director of Production DGF

- met Mr. J. C. Allen, Asian Development Bank/Aquatic Farms

26 July - Traveled to the Padi District: met with Mr. Soetjoko, Chief of Fisheries Department, and Mr. Soewoto, PPL Brackishwater Pond for Batangan area.

- Traveled from Padi to Rembang area met with Mr. Sloch

- Traveled to Sluke Hatchery met with Mr. Bombang Hiwono Hatchery manager

- Returned to Senarang by car and to Jakarta by airplane.

27 July - Traveled to Cirebon and returned to Jakarta

28 July - AM Visited D.G.F. offices in Jakarta. Met with: Mr. Soewito, Director of National Resources Department, and Mr. Willian C. Chan, UNDP Sea Farming Development Project. INS/81/008

28 July - PM Discussed Artemia Development Potential with Drs. Soebekt, Director of PT. CAKRA BHUANA NUSANTARA.

29 July - Met with Mr. Lubus D.G.F. Production Department Director at his house to discuss linkages with the State Salt Enterprise

30 July - Traveled by car to Bogor. Met with Dr. Chhorn Lim R.M.I. nutritionist and continued on to the faculty of fisheries, Bogor Agricultural University, where I met with Dr. Eidman Dean of the faculty.

31 July - AID office discussions with Mr. K. Rishing.

- Met with Mr. Klempim, World Bank/Jakarta

1 August - Traveled by jeep from Jakarta to Chairbon and Eretan with Husini consultant group.

2 August - AID office, met with Ferris Owen, Cooperative League of the U.S.A.

3 August - Met with Soesanto Sahardjo, Director of Programme Development, Ministry of Industry.

6 August - Return to U.S.A.

RECOMMENDATIONS

- Should the GOI proceed with further construction of hatcheries, whether for finfish, Macrobrachim or Penaeus, it is imperative that this development be coupled to in-country brine shrimp production.

To alleviate widespread in-country deficiencies of Artemia a multifaceted approach should be undertaken. Such approaches should include:

A) Political/Financial

1) USAID together with DGF should take political and practical measures to dissolve any import duty or value added tax on Import or Export of Artemia cysts. The 100 percent value added tax or duty on Artemia imports should be repealed as soon as possible. Artemia on the world market is priced at $25.00 USD per 15 oz. can, and is considered a bottleneck to further aquaculture development. In Indonesia poor quality products are often imported at 35-80,000 Rp/500g can, which only aggravates the already difficult situation.

2) Prior to any assistance by USAID the counterpart agency has to demonstrate its ability to account for all project expenditures, perhaps through a private accounting firm.

3) Policy within the AID/DGF and perhaps other counterpart agencies requiring prepayment of expendable items and misc. purchases in

operating budgets should be abolished. Items under $1,000 USD should be purchased by the expatriate long-term consultant, through whatever means available, with accountability for these small expenditures held within USAID or a private accounting agency.

4) Efforts suggested to improve various projects should not duplicate assistance from other donor agencies. Belgium has a 50,000 USD equivalent grant to the DGF to train personnel and construct a demonstration pond in Sampang on Madura Island. Close collaboration with Belgium inputs could greatly assist the successful development of this project.

B) Artemia Pond Production

5) The attached Artemia pond production manual in Appendix V should be translated and distributed to all interested parties.

6) AID assistance to the two demonstration pond units (Bali and Sampang) should be approached with caution. Specific physical modifications are recommended in the appropriate sections. Information on experimental design or management of these units were not available for evaluation. Without a clear-cut experimental design or demonstrated management strategy, no assistance to support activities at these units should be given.

- The Sampang Demonstration pond system has some assistance from the Belgium government. It appears to be underfunded, and any negative

results should be evaluated carefully. Even with expert advice, the project may not succeed without funds to implement good ideas.

7) A stepwise plan to develop in-country production of Artemia cysts should be developed, preferably involving the private sector to the greatest extent possible.

- The State salt enterprise (Perusahaan Umum Garam) has expressed interest in developing Artemia cyst production on Madura Island. A large-scale program to develop Artemia cyst and biomass production should be developed between the State Salt Enterprise and USAID. Ways to collaborate with D.G.F. personnel should be solidified. Technical personnel should receive in-country training in Artemia cyst production.

- The state salt enterprise is presently investing in the reconstruction of Brine Storage Units but this is a slow process, 10-20 Ha/year. Brine Storage Ponds are used to store high salinity water (120-250) during the rainy season. These ponds are planned at each of the 5 salt farms in the East Java, and Madura areas and development at these sites should be accelerated. For example Brine Storage Units in Palmekasan (16 + 18 Ha) and Sampang (10 Ha) could produce as much as 1,760 kg of cysts and a large quantity of adults (biomass) during the rainy season! The adults could be stocked into evaporation ponds at the onset of the dry season.

- Evaporation ponds at the five sites operated by the State Salt enterprise (5,750 Ha total area) on Madura Island and N. East of Surabaya which account for 80-86 percent of the total area, should on an as need basis be dredged to their original depths of 0.45-1.5 M depth. The salt enterprise expressed interest in collaborating to improve these units and would provide some funding and infrastructural assistance.

8) A special program should be undertaken to assist development of in-hatchery production of Artemia biomass and Nauplii. Assistance to this project should be tied to specific results.

9) Methods for decapsulation of cysts, hatching and hygienic use of Artemia need to be translated into Indonesian and distributed to all existing hatcheries.

C) Training

10) Short-term training of three to five technical, not administrative, level personnel at the Artemia Reference Center in Belgium should be supported. This could be arranged through the International Center for Marine Resource Development, ICMRD, at the University of Rhode Island.

11) Two to three technical level personnel should be trained at Artemia cyst producing farms in the U.S., Dominican Republic or Thailand in techniques of pond production and processing.

12) A long-term two-year position for an expatriate should be established to work with the various agencies and private groups on an ad-hoc basis to solve Artemia biomass and cyst production and technical problems. This position would be based in Surabaya, and should take in responsibility for training Indonesian personnel in the large-scale, artisanal and in-hatchery production of Artemia and Rotifers.

13) Indonesian counterparts should be trained in the chemical and biological quality assessment of Artemia at the University of Rhode Island. The person(s) selected would be responsible on their return for setting up an in-country quality assessment laboratory. I would recommend Dr. J. Wirganti, DGF, Jakarta, Mr. A Kahar Rasul, DGF, Semarang and Mr. Mulyono Sastrowidjojo, DGF, Surabaya for these positions. This laboratory could receive samples from all salt farms. Based on the chemical analyses the Artemia batches could be blended to give consistent quality.

APPENDIX I

PENAEID SHRIMP HATCHERIES IN INDONESIA

Province	Name of Hatchery or Owner	Location	Approximate Capacity (Million/Year)
I. Aceli	1. Hatchery Sub-center (government)	Ujung Batee	100,000
	2. Shrimp Hatchery	Uleu-Iheu	100,000
	3. T. A. Hanafiah (private)	Banda Aceh	1,000,000
	4. P. T. Udita Mina (private)	Langsa	1,000,000
	5. C. V. Miranti (private)	Aceh Timur	1,000,000
II. Riau	6. P. T. Hari Baik (private)	Injin Batu, Batam	2,000,000
III. West Java	7. P. T. Gramina Swadaya (private)	Karang Sugra, Serang	40,000,000
	8. P. T. Windu Samudera Mulia (private)	Karang Papak, Sukabumi	6,000,000
	9. C. V. Setia Kawan (private)	Sukabumi	2,400,000
	10. P. T. Udang Mas (private)	Kararig Hantu, Serang	60,000,000
	11. C. V. Langsung (private)		80,000,000
	12. N. V. Padasuka (private)	Serang	180,000,000
	13. Amir Supriyadi (private)	Karawang	90,000,000
	14. P. T. Indeks Udang (private)	Karawang	2,000,000
	15. P. T. Hab & Sons (private)	Cikampek	2,000,000
	16. P. T. Asia Makmur (Private)	Karawang	2,000,000

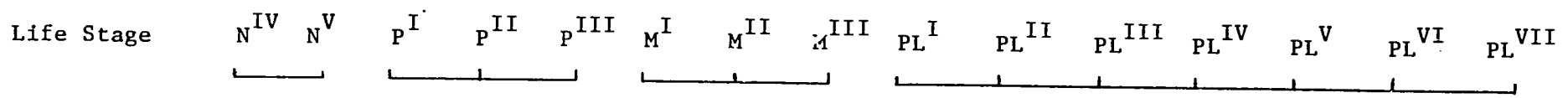
Province		Name of Hatchery or Owner	Location	Approximate Capacity (Million/Year)
IV. Central Java	17.	BBAP (Brackish-water Aquaculture Development Center) (government)	Jepara	864,000
	18.	P. T. Skalatama (private)	Jepara	500,000
	19.	P. T. Fajar Jaya (private)	Jepara	500,000
	20.	C. V. Indera Penaeid (private)	Jepara	500,000
	21.	Bondo Hatchery (private)	Jepara	500,000
V. East Java	22.	Shrimp Hatchery (government)	Situbondo	50,000
	23.	Shrimp Hatchery (government)	Probolinggo	50,000
	24.	C. V. Kembang Sambi (private)	Situbondo	2,000,000
	25.	P. T. Benur Unggul (private)	Besuki	2,000,000
	26.	P. T. Muhara Biru (private)	Situbondo	2,000,000
	27.	U. D. Hendra Jaya (private)	Besuki	2,000,000
VI. South Sulawesi	28.	Shrimp Hatchery (government)	Barru	4,000,000
	29.	P. T. Bonecom (private)	Barru	20,000,000
	30.	P. T. Serdit & Co. (private)	Bulukumba	4,000,000
	31.	P. T. Nursale Galesong (private)	Galesong, Takalar	4,000,000
	32.	P. T. Bina Upaya Benur (private)	Serpok, Pinrang	5,000,000

Province	Name of Hatchery or Owner	Location	Approximate Capacity (Million/Year)
	33. P. T. Izuma (private)	Pinrang	4,000,000
VII. West Kalimantan	34. Johanes (private)	Mempawah, Pontianak	50,000,000
VIII. South Kalimantan	35. Shrimp Hatchery (government)	Kotabaru	200,000
	36. P. T. Misaya Mitra Co. (private)	Kotabaru	5,000,000
	37. C. V. Desco (private)	Tanah Laut	5,000,000
		TOTAL:	581,764,000

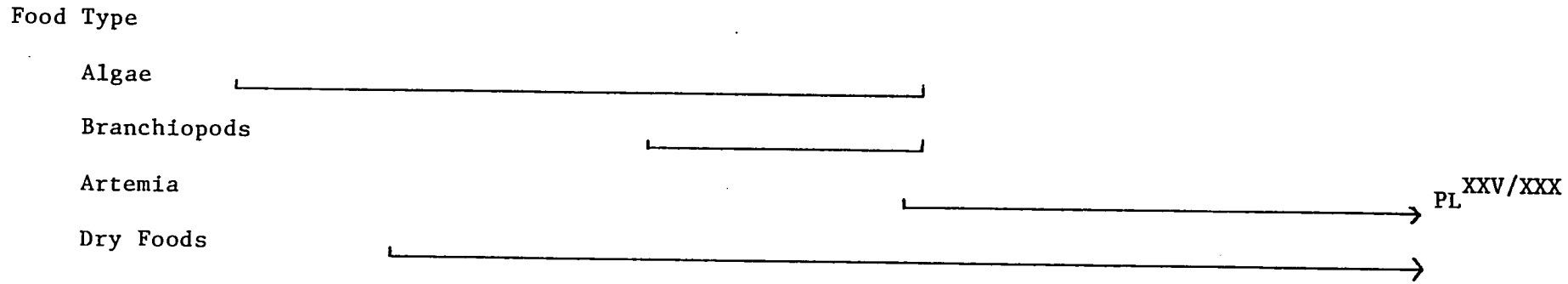
APPENDIX II

APPENDIX II

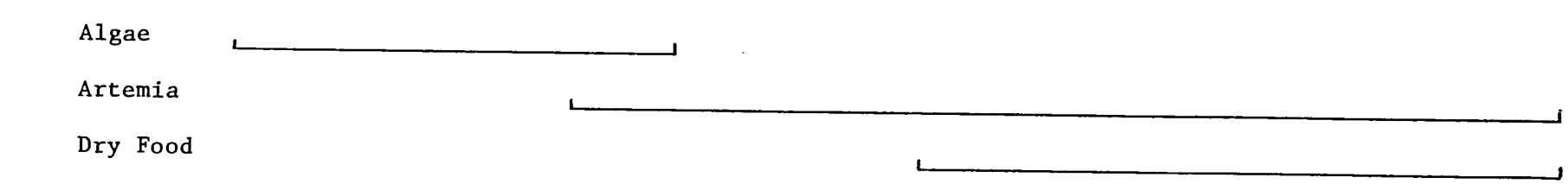
LARVAL SHRIMP FEEDING PRACTICE



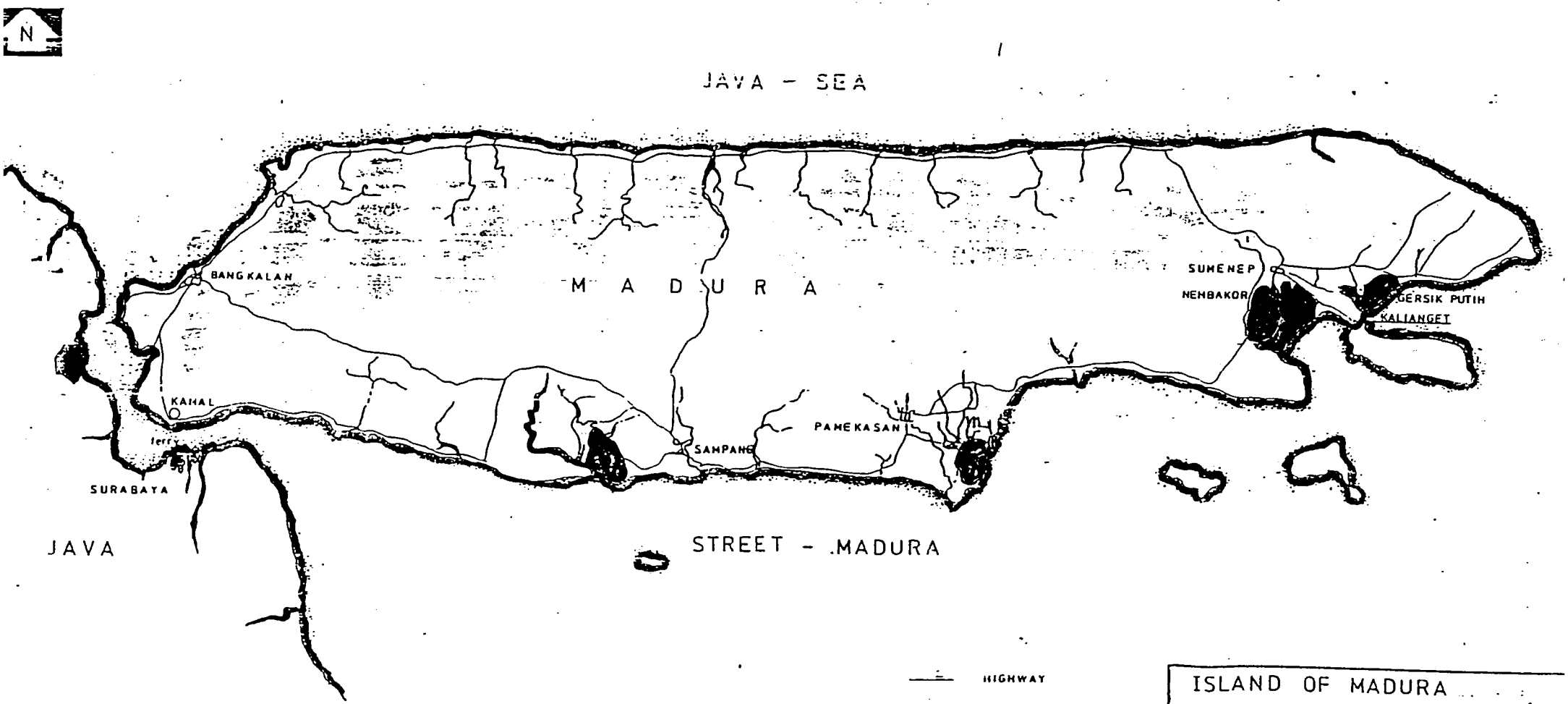
Penaeus monodom at Windu Mas, Bali, Indonesia



Penaeus vannamei, Ralston Purina, Panama, Central America

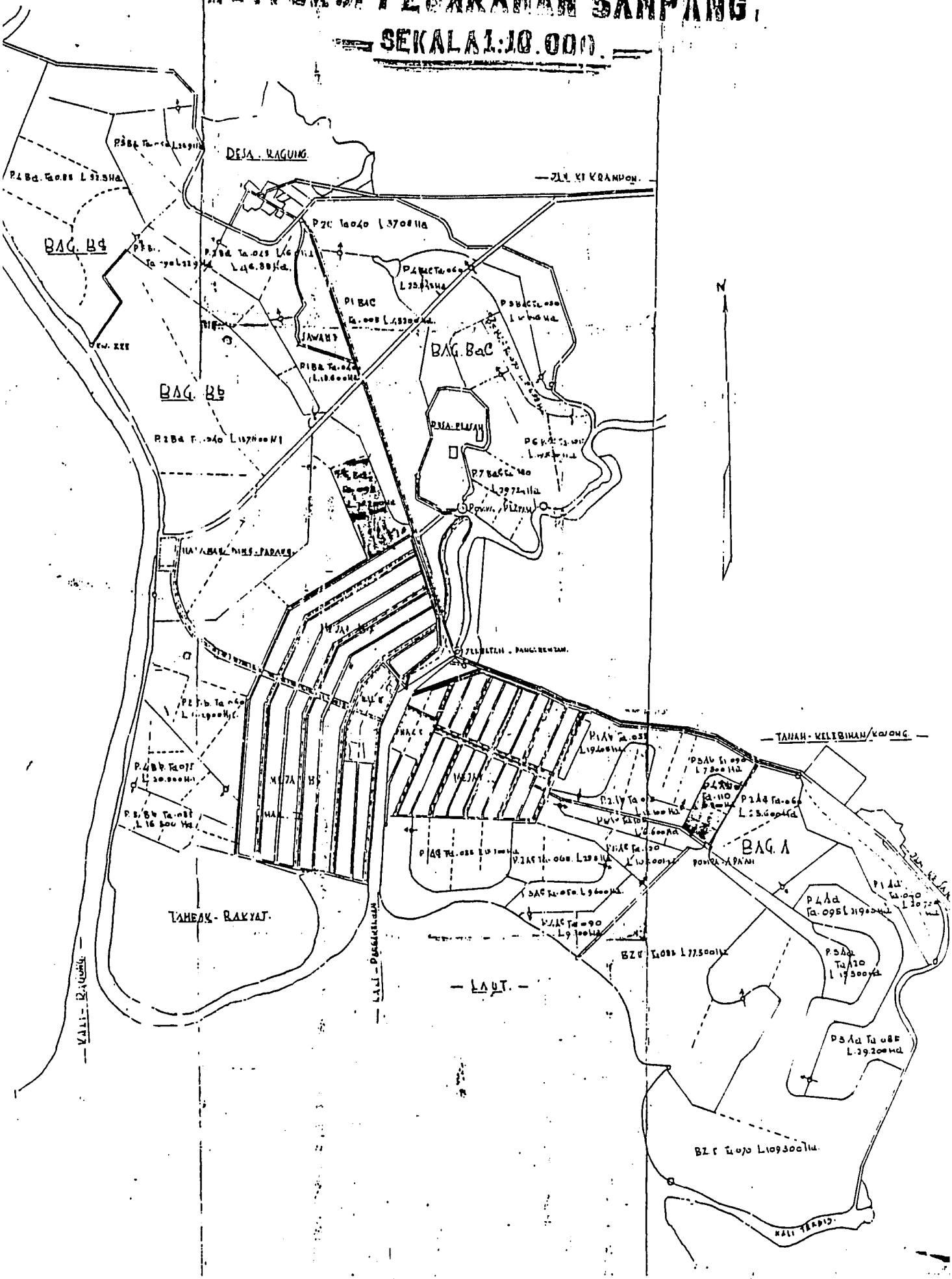


APPENDIX III


N
JAVA - SEA
BANGKALAN
M A D U R A
SUMENEP
NEMBAKOR
GERSIK PUTIH
KALIANGET
KAMAL
PAMEKASAN
SAMPANG
SURABAYA
JAVA
STREET - MADURA
HIGHWAY
SALT FIELD
ISLAND OF MADURA

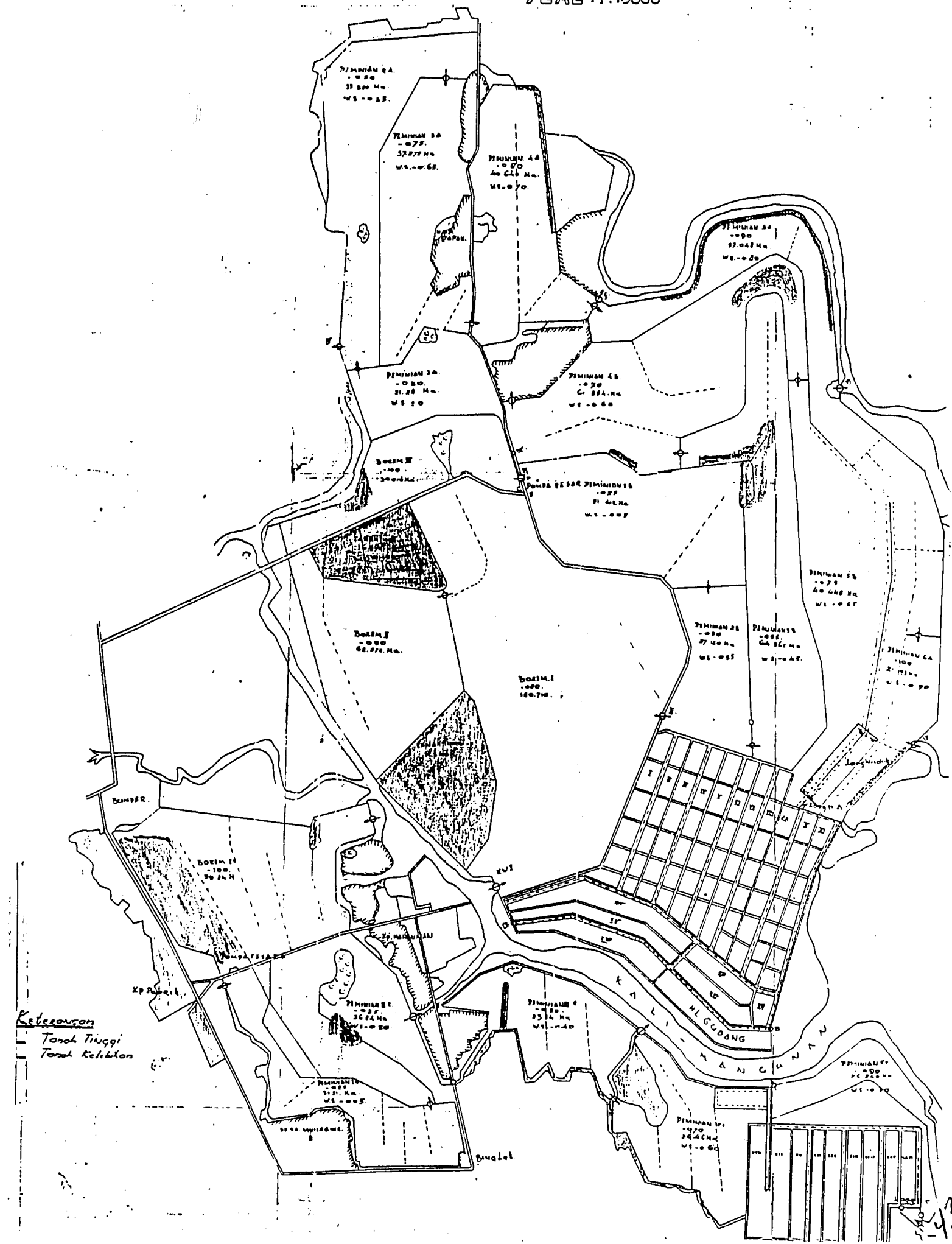
SITUASI PERAIRAN SAMPANG.

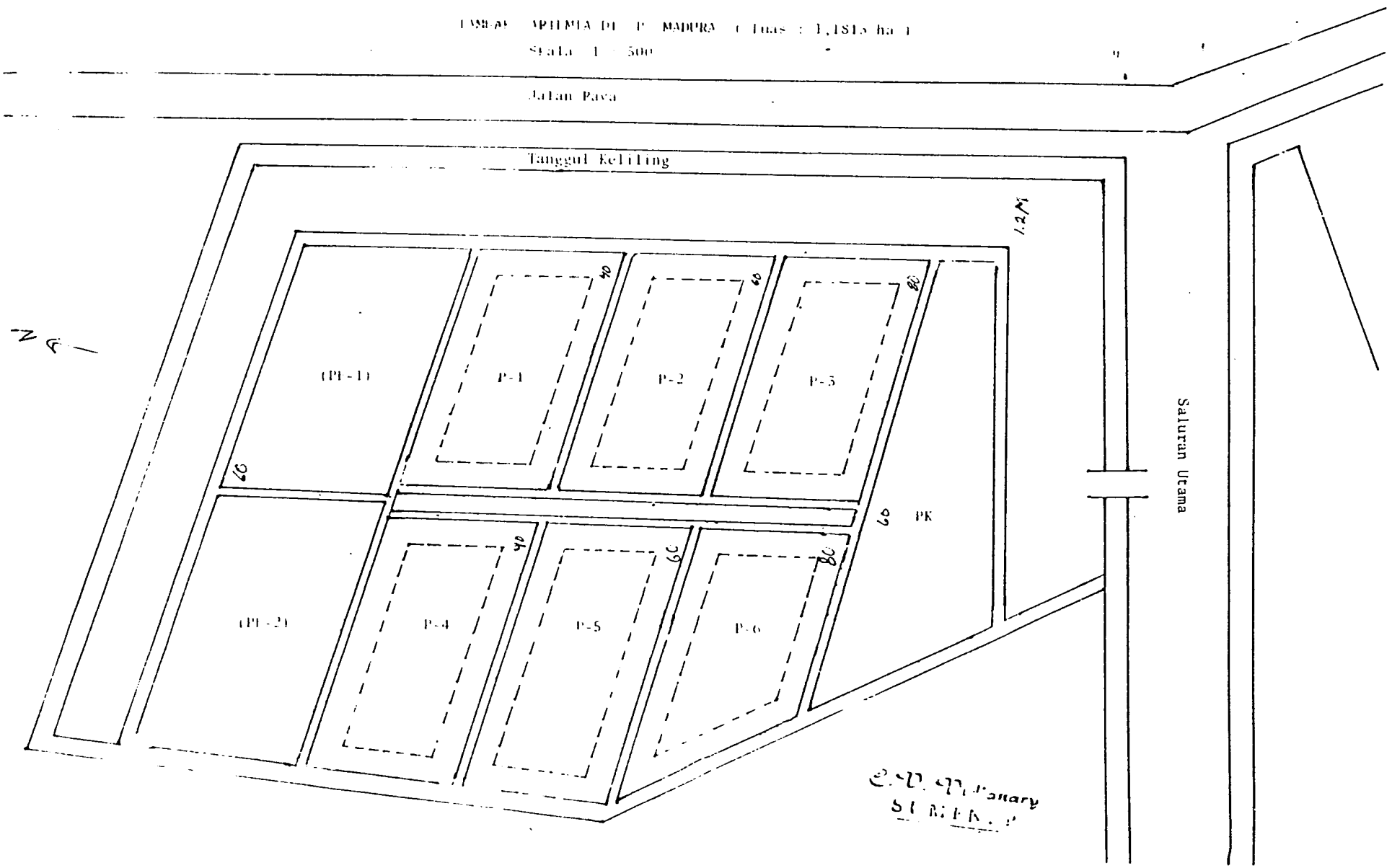
SEKALA 1:10.000



PEGARAMAN PAMEKASAN

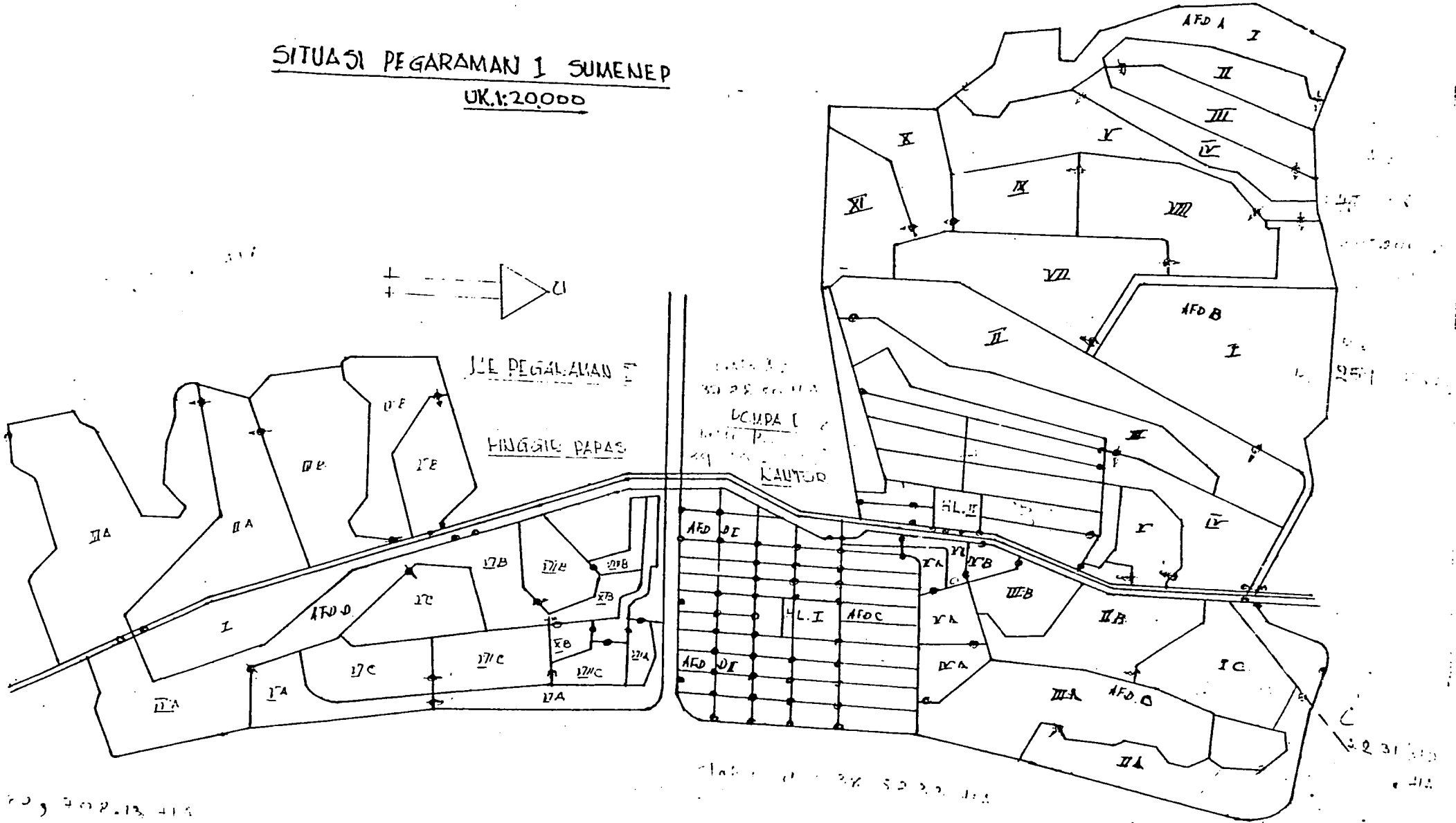
SKALA : 1 : 10000




Jalan Raya
Tanggul Keliling
1.2 M
(PI-1)
P-1
P-2
P-3
(PI-2)
P-4
P-5
P-6
PK
Saluran Utama

SITUASI PEGARAMAN 1 SUMENEP

UK.1:20.000



APPENDIX IV

ARTEMIA CULTURE

INDIA STRAIN

(Mulyono, Subyanto, 1983)

Preliminary experiment:

Experiment I Dr. McVey brought in o/a 200 pairs of artemia (male and female) with salinities 29-31 ppt and were rear in aquarium (40 x 60 x 50 cm size) and feed with rice brand and was aerated.

Experiment II From experience I, was taken 60 pairs and put and raise in different salinity, 30 ppt, 50 ppt., 80 ppt resp and after 60 days the result was as follow:

Salinity	Adult (pairs)	Adult	Nauplii
30	20	500	4,500
50	20	1,200	12,600
80	20	800	8,600

Experiment III This experiment is the follow-up experiment from experiment II and using concrete cement tank, size 4 x 1 1/2 x 1 m and the salinities 50 ppt during 45 days - After o/a 105 days the artemia population was significantly increased up to 400,000 or 100 animals/ltr During Dr. Patrick Sorgeloos visit to Prigi, he suggested to produce nauplii as a stock for feeding the macro brachium rosenbergii.

Feed: 5 kg Rice Brand/day @ 150 Rp/kg

1 kg Yeast/5 days 1,050 Rp

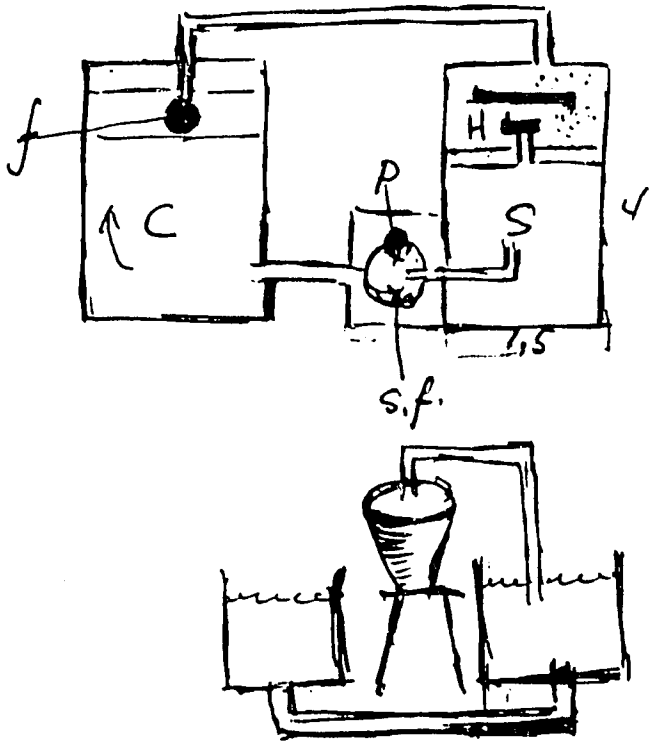
Time: Cleaning 1 hr 8 a.m.

Feeding 8 - 12 - 4 pm - 7 pm - 10 pm

Experiment IV

(closed) covered system for Artemia Culture

with USAID sponsored, we conduct the artemia culture through covered system and to be expected the nauplii production will be increasing.



Remark:

C = Culture Tank

H = Harvest Tank

F = Filter 200 u

S = Sedimentation

P = Pump

SF = Sand Filter

Result of experiment:

- Rice brand, 5 times a day sometimes yeast
- Using Ø 1" pump with a capacity of 1 ltr/sec
- Filter size 200 micron
- 60 days period -
- Artemia densities 5,750 tails/ltr
  Starting 100 tails/ltr
- average nauplii's production 400-600,000/day

Conclusion:

- Closed or covered system for artemia culture should be further developed.
- Difficulty in cleaning out the residue of the food due to high densities need to be explore.

Doc.4092A,p36-37,jh:7/31/84

APPENDIX V

University of Rhode Island, Kingston, RI 02881-0804, U.S.A.
International Center for Marine Resource Development: ICMRD
126 Woodward Hall

BRINE SHRIMP AS A FOOD FOR AQUACULTURE ORGANISMS IN DEVELOPING COUNTRIES

Brine shrimp are uniquely adapted organisms

Brine shrimp (scientific name = Artemia) are small animals that live in temperate and tropical waters in many parts of the world. Because they have no means of defense against predators, their distribution is restricted to those inhospitable areas where almost no other animals can live. The best examples of such areas are the shallow, high-salinity salt ponds or salinas that are used for evaporation in the production of salt.

The brine shrimp have become adapted to life in these ponds in many ways, but the most critical adaptation is the way in which they produce offspring. In times when the salinity is not too high (less than 80 parts per thousand or ppt; normal seawater is about 35 ppt) they produce live larvae, called nauplii. When the salinity increases to the 80-200 ppt range, however, they produce encysted eggs which float to the surface and can later withstand complete dessication in the dry pond bed. The encysted eggs, called cysts, only hatch when the pond refills with lower salinity water.

Larval brine srhimp are the best and most convenient food for larval fish and shrimp

Aquaculturists have found that the cysts can be easily collected from the leeward shore of a salina. Given simple processing, dried cysts can be stored in vacuum cans and hatched at the conveninece of the aquaculturist. Hatching is accomplished by placing the cysts (which resemble brown powder) in regular seawater and bubbling air

50

through the mixture for 24-36 hours. The live nauplii that result, a little less than 1/2 mm in length, can then be used as an excellent living food for the larval fish or shrimp that the aquaculturists is raising.

The world-wide shortage of brine shrimp cysts is a bottleneck in aquaculture development

A serious shortage of Artemia cysts developed during the mid-1970's because aquaculturists began demanding more cysts than the market could supply. In the late '70's, some new suppliers began operations in developing countries and the shortage was temporarily alleviated. At the same time, a collaboration of scientists from the University of Rhode Island and 4 foreign institutions joined to form the International Study on Artemia; their purpose was to assess the nutritional quality, genetics, and biological characterists of brine shrimp from various geographic areas, in response to the increased need of aquaculturists for high-quality live food. The growth of the aquaculture industry, and the aquaculturists' awareness of nutritional quality, has caused the price of high-quality Artemia cysts to rise to approximately $50/kg, due to new supply shortages. Thus, the demands of an expanding aquaculture industry in developed countries has increased the price of Artemia cysts so that the aquaculturists in developing countries cannot afford them.

Proper coupling of in-country brine shrimp production is necessary for successful aquaculture development in many developing countries

Aquaculture facilities which require a metric ton or more per year of Artemia cysts in a developing country often find it difficult to pay $50/kg on the international market. An alternative solution to the problem of cyst availability is the coupling of cyst production

within the developing country. Bearing in mind the potential for product development and export market, it is an especially attractive solution to developing countries' needs where salinas already exist, as well as those where production ponds could be constructed. Developing countries with coastlines in tropical or subtropical areas already have two key ingredients for brine shrimp production: seawater and abundant sunlight. If shallow ponds exist or could be constructed, and if inexpensive organic fertilizers (e.g., manure) are available, it is likely that Artemia cysts can be produced.

Brine shrimp enhance salt production in a salina. Brine shrimp in salinas help both salt producers and aquaculturists.

When the shrimp are not present, the evaporation ponds tend to be dominated by blue-green algae, which produce a mucous material that slows evaporation. When Artemia are present and the ponds receive organic fertilizer, the blue-greens are outcompeted by other kinds of algae that the brine shrimp can eat; thus, no mucous production. Furthermore, the excretory products of the Artemia are carried into the final evaporation ponds and are used there by bacteria which cover the bottoms of the ponds. The deep-red color of those bacteria causes solar heat to be retained in the ponds, enhancing the evaporation process. Biologically-managed salinas are more efficient and also result in brine shrimp cyst production. The salt producer obtains higher quality salt product and can also collect the Artemia cysts for sale to aquaculturists within the country. The adult brine shrimp can also be harvested from the ponds periodically and fed to larger fish or shrimp that are being raised.

The quality of the brine shrimp cysts is critical to their successful use

Research conducted by the University of Rhode Island has shown that the nutritional quality of Artemia depends on their pond environment. The brine shrimp transfer not only nutrients, but also contaminants such as pesticides to cultured fish or shellfish. In controlled tests, this has resulted in very high mortalities of the cultured organisms. Quality of the brine shrimp can be predicted on the basis of chemical analyses. Proper pond management can result in nutritionally good quality cysts; proper processing of the cysts can result in good hatching quality.

Information about Artemia is available to AID from the University of Rhode Island

Technical assistance, information, and training are available to U.S.A.I.D. and the host country counterpart through its Cooperative Agreement with the University of Rhode Island's International Center for Marine Resource Development.

MANUAL ON ARTEMIA PRODUCTION IN SALT PONDS IN THE PHILIPPINES

by

Jan Vos
Associate Expert FAO

and

Nympha L. de la Rosa
Counterpart BFAR

CONTENTS

LIST OF FIGURES

1. USE OF ARTEMIA

.1. Systematic classification

Artemia salina, Cl. Crustacea, Scl. Branchiopoda, O. Anostraca
English name: Brine Shrimp

.2. Occurence:

Artemia naturally occurs in salt lakes and brine ponds, with salinities ranging from 60-300 ppt.

3. Importance

- Artemia, and especially their naupliae, are used in aquaculture as a live food for commercial larval rearing (hatcheries) of many species of fish and Crustacea.

- Other types of animal live feeds exist for these purposes, such as Moina and Daphnia (Cladocera) and Brachionus (Rotifera), but it has been demonstrated on several occasions that Artemia naupliae constitute not only the best, but in most cases the only source of live food available.

- Compared to inert food (pelletized diets etc.) live animal feed has the advantage that it can be administered in larger amounts thus covering a longer non-feeding period, without contaminating the culturing water caused by sedimentation of food particles.

- Compared with the other live food, Artemia has the following advantages:
  - The "eggs" occur in a dry dormant state (cysts) and can be stored for many years. Appropriately packed they can be sent all over the world.
  - From the cysts, live larvae i.e. naupliae, are easily and quickly obtained by hatching them in seawater. Thus, no separate culturing tanks are needed to obtain large quantities of live food.
  - The naupliae survive and remain active in water of different salinities. Even when put in freshwater, mortality only occurs after 2-3 hours.
  - The naupliae have a very high protein content (up to 40%), which makes them an excellent food. Freshly hatched naupliae (Instar I = first larval stage) have a higher nutritional value than 2 or 3 day

1.4. Bottleneck

- Inadequate supply and/or the high price of *Artemia* eggs are the major constraints in the mass propagation of shrimp and prawn in S.E.-Asia.
- S.E.-Asia has no natural occuring *Artemia* sources and is therefore dependent on costly import of cysts.

2. BACKGROUND AND ECONOMICS

2.1. History

-1930-1940: Seale and Rollefsen found that the larvae of Artemia salina were an ideal food for growing fish larvae, and that Artemia cysts could be stored for many years when kept dry.

-Also for other freshwater and marine animals it was found to be a good food.

-1940: First commercial distribution of Artemia cysts harvested from natural finding-places: saltflats of San Francisco Bay and Great Salt Lake (USA).

-1960: With increasing demand, cysts from Saskatchewan (Canada) became available. Total distribution at that moment: a few 10,000 kg, not enough to fulfill demand.

-Monopolization of the market resulted in increasing prices: up to 50 US$ per kg FOB San Francisco.

-In 1978 about 10 commercial distributors of Artemia eggs exist: Buenos Aires-Argentina, Dampier and Shark Bay - Australia, Macau-Brazil (inoculated race), Chaplin Lake-Canada, Aigues Mortes - France, Cadiz-Spain Izmir-Turkey, Great Salt Lake, San Diego, East and West San Francisco-USA. More than 50 tons are harvested annually.

Thanks to this, price went down to about 30 US$ per kg in summer 1978.

-For the moment supply seems to meet demand. Yearly production is more than 100 ton.

2.2. Cost

Right now in the USA and in Australia, good quality cysts can be bought for 20-30 US$ per kg local price. Nontheless prices in countries importing cysts are still high due to taxation, transportation and locally made profit. Thailand: 50-60 US$ per kg. Philippines: 60-85 US$ per kg depending on direct import or through local dealer.

2.3. Natural strains

About ten commercial distributors exist, but many more finding-places of natural Artemia (different strains) have been or are being found all over the world. In Asia the following countries have natural Artemia strains:

China, India, Iraq, Iran, Israel, Japan, Turkey. In S.E. Asia Artemia does not occur because of the distinct heavy rainy season which prevents the existence of permanent brine ponds or lakes.

2.4. Inoculation

Since 1977, Artemia has been successfully inoculated in lakes or pond systems which did not have natural Artemia.

Macau Brazil: In April 1977, a 10 - ha salt pond was inoculated with naupliae of the San Francisco Bay Brand strain hatched out of 250 g of cysts. In June of the same year the first batch of cysts was harvested and the Artemia population spread out over the entire salt pond system (area of about 1000 ha). From then cyst production increased sharply. December 1977: 6 tons of cysts harvested, November 1978: 25 tons, 1979: more than 40 tons.

Barotac Nuevo Philippines: February 1978, 125,000 pre-adults and naupliae hatched out of 80 g of cysts of the San Francisco Bay Brand strain were inoculated in a 300 m^2 concrete tank containing brine. After feeding them, they were transfered to two saltponds with a total area of about 6,500 m^2. At the end of May 1978, a total of 35 kg of good quality cysts plus 30-40 kg wet weight of adults were harvested.

Chachoengsao-Thailand: Inoculation experiments started in 1978-1979 in transformed evaporation ponds in saltfarms. In 1980 more than 250 kg of cysts were harvested.

Mundra and Goa-India: Inoculation tests were successful end 1978.

3. BIOLOGY

3.1. Different strains

More than 50 different strains from all continents exist with differences in specific characteristics such as hatching rate, nauplius size, viability, optimal temperature and salinity range requirements.

3.2. Morphology and life cycle

For morphology see practical work sheets and Fig. 1

- Dry eggs or cysts (0.2 - 0.3 mm) hatch in seawater into free-swimming naupliae (0.45 mm) in a period of 24-36 hrs.
- Depending on feeding conditions, the naupliae will grow out to full-grown adults (max. 12 mm) in 1 to 3 weeks time.
- Males and females are easily distinguishable and the female will produce offspring after copulation with a male. (only in zygogenetic strains). Prior to copulation the male will ride the female (riding position) for a period of a few hours to several days, by holding her with his graspers. At a certain moment the male will curve its body and insert its paired penis in the uterus aperture at the bottom of the broodpouch upon which the eggs are fertilized.
- The offspring will be either in the form of cysts or directly free-swimming naupliae (see reproduction).
- In the case of cyst offspring, the female deposits the cysts but they will only become hatchable after being dried.
- During the drying process (dehydration) the spherical shape of the deposited eggs changes into the shape of a crunched ping-pong ball (dented).
- Once in the dried state the cysts can be stored for later use.

3.3. Reproduction

- Some strains are parthenogenetic (only females) but most are zygogenetic (males and females).
- Two modes of reproduction exist in Artemia:

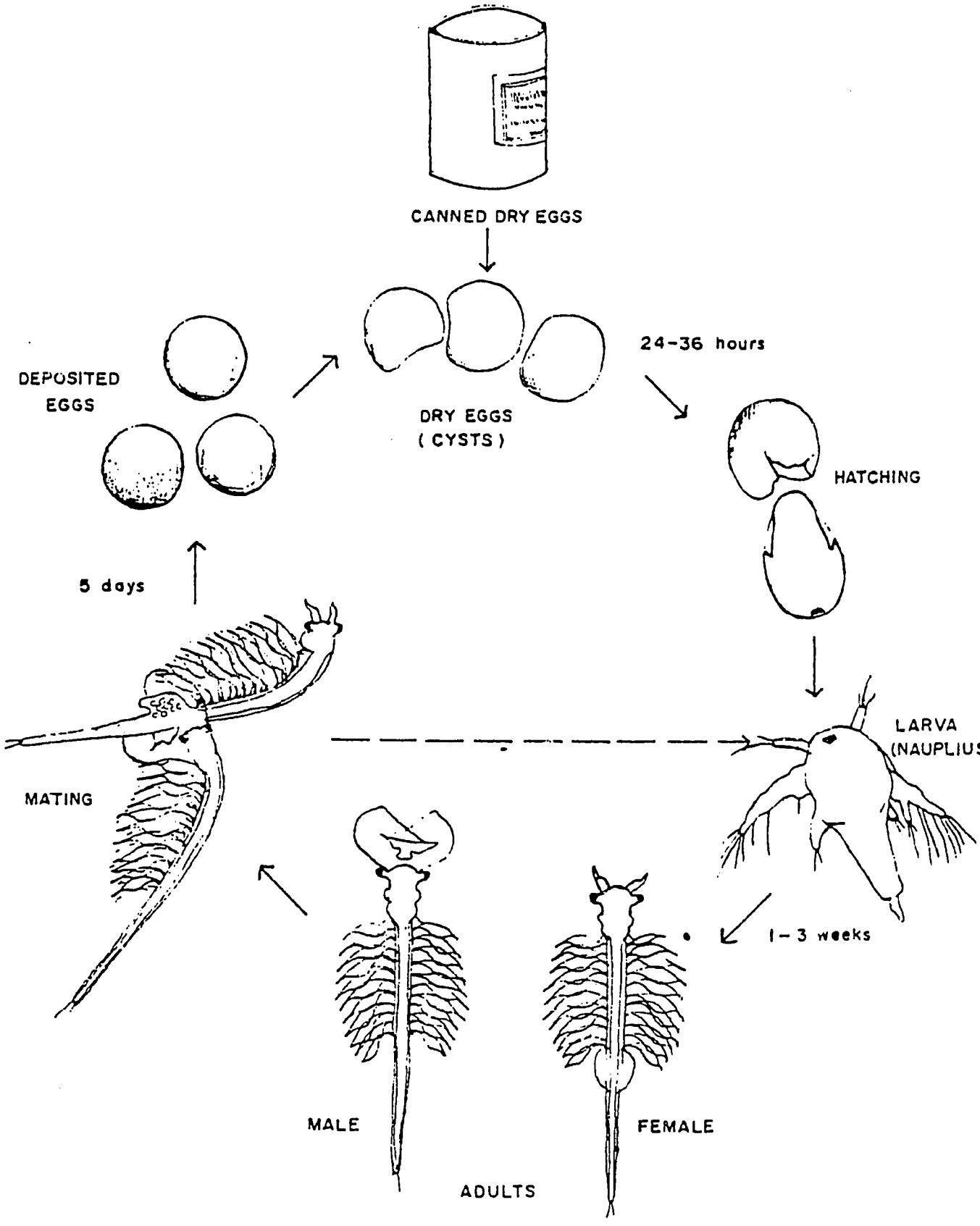


Fig. 1 LIFE CYCLE OF THE BRINE SHRIMP (ARTEMIA SALINA)

Oviparous reproduction; After copulation fertilized eggs are surrounded in the broodpouch of the female with a tough brown shell. The egg is then called a cyst. The cysts are released by the female in the water where they will not hatch untill they have been completely dehydrated (in nature by floating ashore and sun-drying). The embryo inside each cyst is then in a state of metabolic dormancy and will not further develop until hydrated again (water absorption). In nature this mostly happens when washed back to the pond water after rainfall. Once sufficiently hydrated the embryo further develops into the instar I larva (nauplius) which will hatch out of the cyst shell.

Ovoviviparous reproduction: After fertilization the eggs are not surrounded by a shell but instead immediately develop further into naupliae in the broodpouch of the female. These naupliae are then released in the water as free-swimming naupliae.

The mode of reproduction is controlled by environmental factors such as oxygen content and/or fluctuation thereof, type of food and reproduction history of the female. In practice the following assumptions are valid:

Oviparous	Ovoviviparous
- low O_2-content (such as in high salinity)	- High oxygen content (such as in low salinity)
- Strong O_2-fluctuations	- Minor O_2-fluctuations
- Fe-rich food (such as green algae)	- Fe-low food (such as organic debris)

mode of reproduction is determined once the eggs have descended into the broodpouch and are fertilized.

In one batch of eggs produced in a female all eggs are either cysts or ovoviviparous eggs. In other words only one reproduction mode occurs per batch.

Adult Artemia can live for several months (in good conditions) and the female produces a new batch of eggs every 5 days.

Per batch or reproductive cycle 50-200 cysts or naupliae are produced but in oviparous reproduction the number of offspring is generally lower

Cysts

3.4. - Dehydrated cysts of most strains measure between 200 and 270 µm, and weigh 3.5 µg on average.

- Dry cysts are very resistant to extreme conditions. Up to 80°C, hatching efficiency is not affected. Hydrated cysts are killed by temperatures lower than 0°C and higher than 40°C.

- Cysts are very hygroscopic and absorb even water from the atmosphere. When stored, water content should be lower than 0.09 g H_2O/g cyst or 10% (no metabolism). At 0.3 g H_2O/g cyst or 25% the metabolism of the dormant cyst starts.

- At salinities higher then 70 ppt cysts can not hatch because of the too high osmotic gradient. In salinities lower than 5 ppt cysts will hatch but resulting naupliae will die quickly.

- Light triggering is needed at the beginning of the hatching to start metabolism.

- In freshwater and seawater, dehydrated and hydrated cysts sink. In brine (saturated salt solution) they float.

3.5. Naupliae

Growth is optimal at 28°C and 35 ppt and drops below pH 7.

- Lethal temperature limits are 0°C and 37-38°C.

- Salinity changes can be administered very abruptly without harm. For instance from 30 to 90-100 ppt.

- Sudden transfer from 30 to 0°C can also be done without killing them. At 0°C, activity will stop but can be reactivated by increasing the temperature.

3.6. Adults

- Mostly salinity tolerance is up to 200-250 ppt. Limitation is more caused by oxygen depletion than by salinity itself.

- Below pH 7 general appearance of *Artemia* deteriorates. pH 8-8.5 is optimal.

- Lethal temperature limits are the same as for naupliae.
- Just as naupliae, adults can stand sudden temperature fluctuations.
- Low oxygen concentrations are more harmful for young naupliae than for older larvae and adults, since during larval development the exopodites become functional as respiratory structures.
- The colour of adult Artemia is correlated with the mode of reproduction because hemoglobin synthesis is activated by low oxygen concentration in the water. Red Artemia indicate oviparous reproduction, pale whitis Artemia indicate ovoviviparous reproduction.

3.7. Feeding

- Artemia is an obligatory non-selective particle filter feeder and removes suspended particles smaller than 40-60 µm down to a few µm from the water with great effectiveness.
- Food particles may consist of algae cells (non-filamentous), Protozoa, organic detritus particles, etc.
- Only from instar II and III larval stages food is taken up. Instar I through III larvae have a yolk reserve.
- In most waters where Artemia occurs naturally, no other plankton feeder is present. Therefore the only food competition will be among the Artemia themselves.

3.8. Phototactism and distribution

- Young naupliae are positively phototactic.
- Adults are negatively phototactic.
- Artemia tends to gather in clouds along the shores. Sometimes long Artemia trails can be seen in the water.
- Vertical and horizontal distribution patterns are completely different during day and night time.

4. INOCULATION IN GENERAL

4.1. Definition

As applied to Artemia, inoculation means the introduction of the species in a suitable aquatic environment where for some reason the animal is absent.

4.2. Principle

- Inoculation is done with a small number of living Artemia, either in the early nauplius - or later subadult or adult stage. When the environment is suitable in respect to salinity, temperature and food availability, the inoculated or newly introduced Artemia will thrive and reproduce. The population will grow and reach a size that can be sustained by the environment (carrying capacity).

- Mostly food availability is the limiting factor for population growth. Salinity and temperature being within tolerance ranges, an increase in available food will result in a higher carrying capacity and thus in a larger population. A decrease in available food will in the same way result in a smaller population. Since in outdoor ponds the carrying capacity can not be kept constant, population will fluctuate in accordance with the carrying capacity.

  In the case of Artemia, population growth is not density-restricted in unlimited conditions.

4.3. Strain selection

- Artemia has a worldwide (cosmopolitan) distribution. As previously mentioned it occurs in all continents and consequently many different geographical strains exist. Each of these strains have in the course of time become more and more adapted to specific local conditions. For instance concerning temperature, Canadian and Indian strains differ significantly in their temperature tolerance, since they live in almost opposite climatic conditions.

  It follows that an inoculation will have a better chance to succeed if a strain is selected with a habitat as identical as possible to the new site. For the Philippines this means that inoculation strains from other tropical or sub-tropical regions are favorable compared to temperate strains.

4.4. Time of inoculation

- The main reason why Artemia does not occur naturally in S.E. - Asia is because of the heavy rainfall during the rainy season. During this period salinity in every closed water body is washed out and turns into freshwater. Artemia does not survive in freshwater and even if it would, invading predators such as fish, shrimp and water insects would easily eradicate the population.

- In their natural finding-places Artemia can survive because it occupies a very specialized habitat, i.e. very saline water bodies where only Artemia and a few algae and bacteria species can live. Predation is therefore almost non-existent.

- In S.E.-Asia highly saline waters exist only during the dry season and are mostly found in solar salt farms. In these regions, Artemia inoculation should therefore be performed in the beginning of the dry season as soon as a salinity of 100-110 ppt is reached which prevents any kind of predator. At the end of the dry season the Artemia population will die off or be eradicated completely. A new inoculation will be required in the beginning of the following dry season.

  In countries with a negative yearly water balance and no distinct heavy rainy season, inoculation has only to be performed once to establish a permanent Artemia population.

5. POND PREPARATION

5.1. Pond design

Once a suitable pond is selected it should be prepared for inoculation. Concerning design this might require:

- Deepening of the pond. Water depth in the pond should be 40 cm or more to prevent too high temperatures. If the selected pond is too shallow it can be deepened either by excavation, by heightening the dikes, or by a combination of both i.e., partly excavation to heighten the dikes resulting in an inner perimeter trench.
- Preparation of water intake structures. These can be ordinary fishpond gates or culvert pipes through the dikes. Whatever water control system is used, it should allow water intake at regular intervals, and for Artemia this is more often than for fishponds.
- Preventing of leakage and seepage through dikes and pond bottom. Soil should be clayish and not sandy. Leakage in newly constructed ponds can be prevented by using hollow blocks in dike construction, concretizing or coring dikes.

5.2. Water intake

Inoculation must be done when salinity of the water is 100-110 ppt to prevent fish and other predators. The salinity in the pond can be increased by gradual evaporation, but this will take a long time (3 ppt/day in summer) and therefore the higher the salinity of the intake water the better. Source can be a saltfarm (from the evaporation ponds) or fishpond water with high salinity. To save time, initial water salinity should not be less than 70-80 ppt. Screen water intake, not only to prevent fish from coming in, but also to prevent entrance of fish larvae which might grow to bigger fish in the pond. The screen used should not be larger than 1 mm mesh.

If no water control structures such as gates or culvert pipes are available, water can be taken in by pump.

Water orginating from mangrove areas is better than straight sea water sources. Mangrove water has a much higher food content for Artemia through its suspended organic detritus particles.

5.3. Fertilization

Upon inoculation, enough food should be present in the water to guarantee good survival of the introduced Artemia. If the water has a natural turbidity of 40 cm or deeper the pond will have to be fertilized to produce a phytoplankton bloom (green water). The following inorganic fertilization is prescribed:

- use a combination of 16-20-0 (monoammonium phosphate) and 33-0-0 (ammonium nitrate) in equal amounts, to provide a high nitrogen base fertilization.
- total application at a rate of 100 Kg/ha and 50 kg/ha of each product.
- inorganic fertilization is done according to pond surface and independent of actual water depth.
- the deeper the water, the better for phytoplankton production. If water is too shallow the fertilizer will stimulate lab-lab growth. Two or 3 days after fertilization a phytobloom of algae species resistant to high salinities (more than 100 ppt) will develop. Then inoculate. If a faster reaction is required (within 24 hrs), dissolve first the 16-20-0 in a plastic bucket of water and let stand overnight. Then broadcast. 33-0-0 can always be broadcasted dry since it easily dissolves.

If no inorganic fertizer is available, use organic fertilizer such as chicken manure at a 0.5-1 ton/ha rate. Contrary to lab-lab production, first take in water and then broadcast the manure.

5.4. Prevention of lab-lab

Contrary to fishponds, lab-lab is not desirable in Artemia ponds. It is not a good food for Artemia and when it starts floating it can seriously mess up cyst harvest. To prevent lab-lab from growing do the following:

- Dry and rake the pond bottom prior to water intake and remove all traces of decaded lab-lab.
- Do not fertilize with inorganic or organic fertilizer before water intake.
- Take in water with high salinity to reach suitable inoculation salinity in a minimum of time. The longer it takes to reach this salinity, for instance through gradual evaporation, the more lab-lab will have a chance

- Have a high water depth in the pond at all times, if possible more then 40-50 cm, to prevent light penetration to the bottom.
- Maintain a high turbidity either by intake of turbid water or by fertilization.

6. INOCULATION TECHNIQUES

6.1. Naupliae or adults

Naupliae as well as sub-adults or adults can be inoculated. Inoculated naupliae will first have to grow to the adult size before population growth can start. Sub-adults inoculation will result in faster effect and is safer in cases where especially temperature conditions are on the tolerance limit (36 - 37°C). However adult inoculation requires first high density culturing in separate tanks.
Inoculation with cysts will not work since cysts can not hatch in salinities of 100-110 ppt.

6.2. Inoculum preparation

- After determination of the number of naupliae needed, the corresponding amount of cysts to hatch can be calculated from the hatching efficiency of the used strain. (see practical work sheets). The hatching efficiency is the weight of cysts (gram product) needed to produce one million naupliae.
  Naupliae inoculation densities are usually in the range of 30- 100 naupliae per liter pond water. This allows some mortality of the naupliae after inoculation.

- Hatching of the naupliae is done according to the instructions in the practical work sheets.

- If inoculation can be performed at the inoculation site itself (electricity available or battery-operated aerators), no special transportation methods have to be used. Naupliae can be carried to the pond in densities half the hatching density.

- After hatching transfer the naupliae to containers with the same salinity as the pond water. They survive the shock. For adults salinity change should be done more gradually.

6.3. Transportation

If hatching can not be done at the inoculation site, naupliae will have to be transported in the following manner:

- Collect and sieve naupliae, and transfer them to the appropriate

- Rinse with 0-4°C salt water and transport them in cold brine at a density of 10 million/20 liter (equivalent to 50 g cysts/20 liter). Motoric activity will stop and they will tend to sink to the bottom. Therefore provide slow aeration from the bottom of the container to keep the animals in suspension. Use styrofoam and ice to keep temperature low, during transportation. Instead of containers, also plastic bags inflated with oxygen can be used.
- Adults and sub-adults can be transported in the same way at densities of 20,000 animals per liter.

6.4. Actual inoculation

- The best time of the day to inoculate a pond is during late evening since at this time water temperature is low and will continue to drop till early morning. If this is impossible, inoculate in early morning.

- Although naupliae as well as adults can be transferred directly from their low temperature transportation containers into the inoculation ponds, it is safer to first let temperature in the containers rise a little so that the animals can resume motoric activity.
- The first days after inoculation it might be very difficult to find or see any of the inoculated naupliae. They lost their distinctive orange color (no more yolk) and they tend to gather in corners of the pond. When inoculation is successful, the first sub-adults should be seen swimming after a week or less.

7. POND MANAGEMENT

7.1. General principle

The following management scheme should be followed in principle: (Fig. 2)

PREPARATORY PHASE

- fill pond with high salinity water and if necessary let further evaporate.
- the pond is ready once 100 -110 ppt salinity with 30 - 40 cm waterdepth is reached and no more predators are present.
- fertilize if not enough food is present in the water i.e., turbidity more than 40 cm. (see practical work sheets)

INOCULATE

GROW OUT

- keep salinity within the 110 - 120 ppt range by regular water intake (see practical work sheets) to let grow the inoculated Artemia to the adult size.

OVOVIVIPAROUS PHASE

- still maintain 110 - 120 ppt salinity range after Artemia have grown adult to let population increase through ovoviviparous reproduction. On their turn first generation naupliae will also grow adult. Population should reach a density of 40 or more individuals (adults plus naupliae) per liter.

SALINITY INCREASE

- let salinity go up to 150 ppt through evaporation, but always maintain sufficient waterdepth.

OVIPAROUS PHASE

- maintain the 150 ppt level by regularly taking in water. The higher salinity will induce oviparous reproduction and thus a large part of the population will start producing cysts.
- keep this going as long as the weather allows it, to keep harvesting cysts.

7.2. Details

- The regular water intakes have three purposes: (1) to not let salinity go up too high. Salinities higher than 150 - 160 ppt are not necessary for good cyst production and might endanger survival through oxygen depletion. (2) To maintain sufficient waterdepth to prevent too high water temperatures. (3) To take in new food in the form of algae and organic detritus particles.

- Regular observations of waterdepth and maximum daily water temperature (2-3 PM) should be carried out. Waterdepth should at all times not be less than 30 cm and water temperature should be lower than 38°C.

- If the water taken in has a low food content (clean, not green water), it is necessary to fertilize in one of the following manners:

  inorganic: 50 kg 16-20-0 and 50 kg 33-0-0 per ha once a week (see practical work sheets).

  organic: 0.5 ton chicken manure once a week.

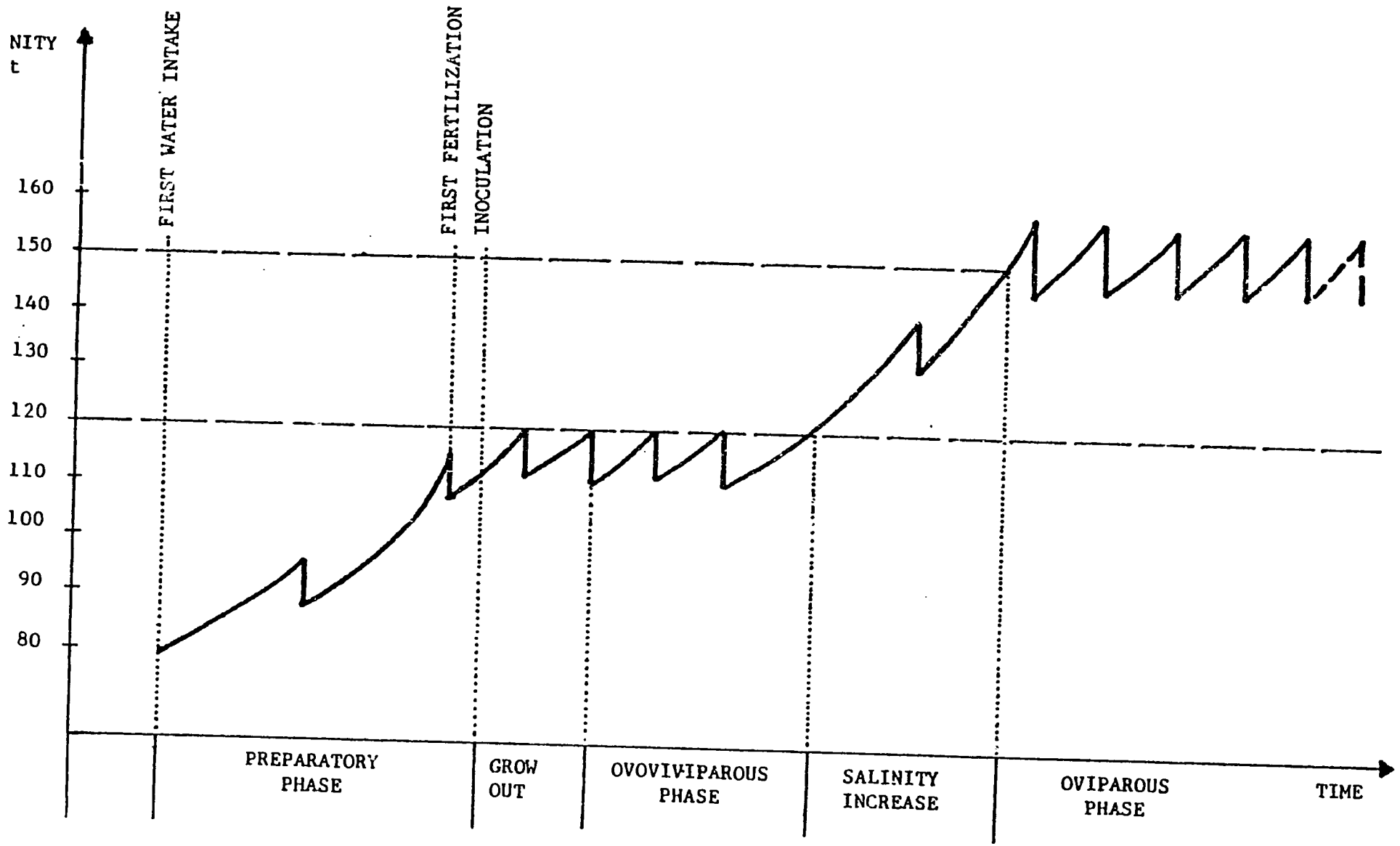
  These fertilization rates depend on local conditions and can be decreased as long as a sufficient high food level is maintained in the water.

- The above described management scheme is theoretical in nature and is merely meant as a guideline. Many things can happen such as:

  - Unexpected rainfall decreasing salinity. A short shower will not affect salinity too much. The danger lies in the formation of a freshwater layer on top of the highly saline pond water. This will heat up the water. There is no problem if after a shower the wind mixes the water layers.

  - No real control on the amount of water taken in, resulting in strong salinity fluctuations. As long as salinity is kept within a 110 - 160 ppt range this should not be a problem.

  - Incapability of taking in water regularly, resulting in too shallow waterdepth. This is the worst that can happen and must be prevented at all times.



: WATER INTAKE

Fig. 2. THEORETICAL MANAGEMENT OF ARTEMIA POND

8. HARVEST PROCESSING

.1. Regular harvests

- Cysts must be harvested regularly from the pond. Several dehydration - hydration-dehydration cycles, which happen when the cysts are left for a long time in the water and floated ashore, will decrease cyst viability and consequently cyst quality. In practice they should be harvested every time a patch of cysts has gathered in a corner and in a good *Artemia* pond this might be daily. Further processing of the cysts can be done once a large amount has been collected. In the meantime the cysts must be stored in a brine container (see practical work sheets).
- Floating cysts will gather in a corner of the pond by wind action. From there they can be easily scooped out with a fine-meshed screen. Make sure the slopes of the dikes in all corners of the pond are steep. This will prevent the cysts from clinging to the soil. Eventually, corners can be lined with plastic sheet to obtain very clean harvests. If floating lab-lab is present, wide-meshed barriers can be placed in the water to keep the corners clean.
- Also a part of the *Artemia* population can be harvested from the pond. Adul *Artemia* can be used as live food for nearby fish and shrimp nursery ponds.

8.2. Processing of cysts

- The processing of the cysts is of crucial importance with regard to the quality of the product. Not only have the cysts to be dehydrated to a sufficient low level, but the quality of the product is also determined by the effective removal of dirt from the cysts. Although many different procedures exist, they all go through the following three steps: cleaning, dehydration, packaging.
- The recommended procedure described in the practical work sheets is based on the bi-phase floatation principle: fractions are separated in solutions with different specific gravity.

8.3. Production potentials

Previous inoculation trials in Thailand and the Philippines indicate a production potential ranging between 25-55 kg cysts/ha/5 months dry season, depending on the use of extensive culture techniques with only mangrove water intake as food source or intensive techniques with fertilization.

These figures are conservative estimates and production can be probably much higher depending on the initiative of the farmer. Nonetheless they already compare favourable with production data of natural *Artemia* populations which average at 20 kg cysts/ha/ 4 months.

8.4. Cyst quality analysis

- The processed cyst product should be checked for its quality. Quality control is easily performed by routine determination of hatching efficiency (HE) and/or hatching percentage (HP). They do not however represent the same thing. Procedures are described in the practical work sheets.

- In the past most cyst distributors used HP as quality criterium i.e., the percentage of cysts that will hatch. This can be very misleading since a commercial product may contain not only cysts but also dirt in the form of sand and detritus, and this is not accounted for in the HP criterium.

- As *Artemia* is sold on a weight basis, a much more realistic criterium is therefore the number of live naupliae the customer will get from a certain quantity of product purchased (HE). The following figures clearly demonstrate the difference between HE and HP and the possibly misleading character of the latter:

	(HP) Hatching percentage (%)	(HE) Hatching efficiency (g)
Brand 1	82	17
Brand 2	87	4.5

According to the HP the brands have more or less the same quality, but the HE indicates that brand 2 is much better than brand 1.
It is therefore recommended to use HE instead of HP as quality criterium, if the necessary equipment is available.

9. SITE SELECTION

9.1. Criteria for suitable sites

1. Availability of high salinity water.
   A source of at least 70 ppt is essential. Sources can be:
   - evaporation ponds in saltfarms. Run-off water from crystallization areas is disrecommended for toxicity reasons.
   - highly saline water from fishponds in late summer.
2. Sufficient water depth.
   At least 30-40 cm depth is necessary to prevent too high temperatures.
3. Regular water intake possibility.
   Once every week or two weeks, without hampering operation of non-Artemia ponds.
4. Seepage and leakage free ponds.
   To be able to maintain salinity and waterdepth.
5. Pollution and contamination free water.
   Agricultural pesticides can bio-accumulate in produced cysts, thus making their hatched naupliae toxic for cultured species.

9.2. Climatology and facilities

- Since high salinity water is needed it is logical to look for sites with saltfarm facilities. Ordinary fishponds, not neighbouring to saltfarms, are unsuitable since high salinity will have to be reached through time-consuming evaporation. Consequently three kinds of sites to consider are:
  - saltfarms Deep evaporation ponds or modified shallow evaporation ponds.
  - fishponds next to a saltfarm with possibility to draw brine from the evaporation ponds of the saltfarm.
  - Newly to develop plots of land situated in a suitable climatic type. To be developed as combination of evaporation area and Artemia ponds with the first area providing brine to Artemia ponds.

- In the Philippines, saltfarms using the evaporation pond system are to be found in Pangasinan, Bulacan, Bataan, Metro-Manila (Las Piñas and Parañaque), Cavite, Iloilo, Mindoro Occidental, Negros Occidental, Bohol, Cebu, Misamis Oriental, Zamboanga, and South Cotabato.

- Not all these areas have climatic type 1 weather, which is the climate with the longest dry season. The longer the dry season the better for cyst production. Following the IRRI agroclimatic map, duration of the dry season in the different saltfarm areas is as follows:

Zamboanga) S. Cotabato)	more than 6 dry months*
Pangasinan) Bataan) Bulacan) Metro Manila) Cavite)	5-6 dry months*
Iloilo) Mindoro Occ.) Negros Occ.) Negros Or.) Bohol) Cebu) Misamis Or.)	2-4 dry months*

* a dry month has less than 100 mm rainfall.

- Because of the traditional lay-out of most saltfarms (shallow evaporation ponds), a lot of saltfarm areas offer not-directly suitable Artemia ponds and will consequently need modification or development. Directly suitable ponds were found in Bohol, Negros Oriental, Iloilo and Pangasinan.

10. PRACTICAL WORK AND EXERCISES

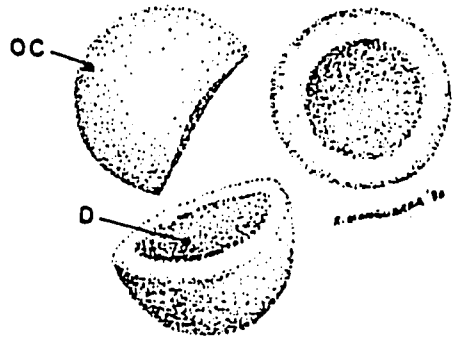
on

Artemia Production in Salt Ponds

PRACTICAL WORK
LIFE CYCLE AND MORPHOLOGY

DEHYDRATED CYSTS (40 x)

Fig. 3

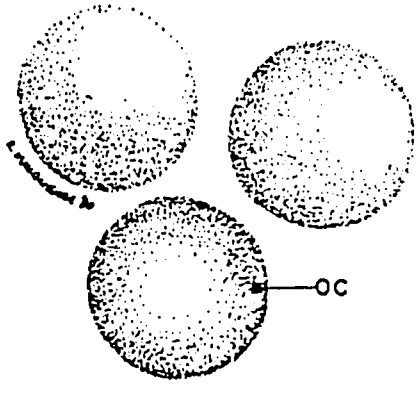


OC. outer cyst shell (color variates from grey-white to dark brown), D. dent caused by dehydration.

Note debris in cyst sample. Debris includes broken and empty shells, sand, feathers, parts of Artemia skeletons etc.

HYDRATED CYSTS (40 x)

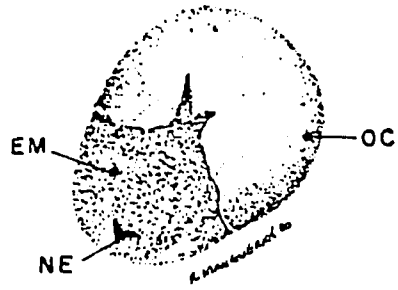
Fig. 4



OC. outer cyst shell.

Dent [illegible]

HATCHING CYST (40 x)



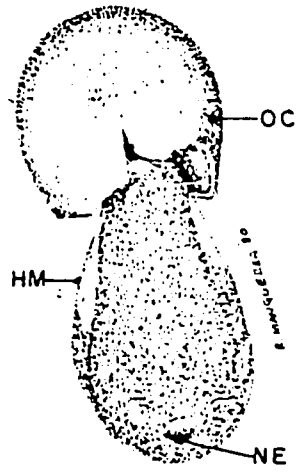


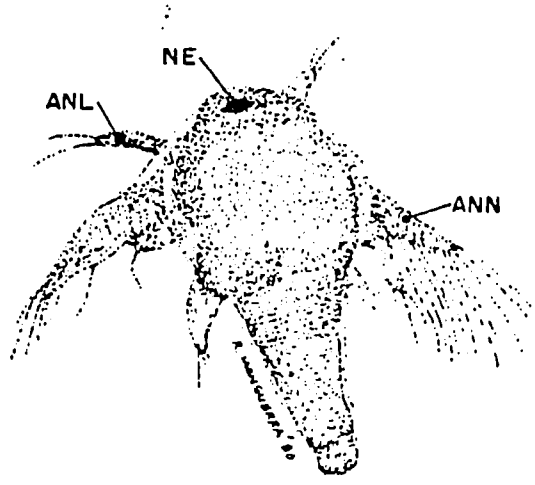
Fig. 5 breaking stage

Fig. 6 hatching stage

OC. outer cyst shell, EM. embryo surrounded by hatching membrane, NE. nauplius eye, HM. hatching membrane.

HATCHED NAUPLIUS INSTAR I (40 x)

Fig. 7



NE. nauplius eye, ANL. antennula, ANN. Antenna.

GROWING LARVA (40 x)

(Instar IV)

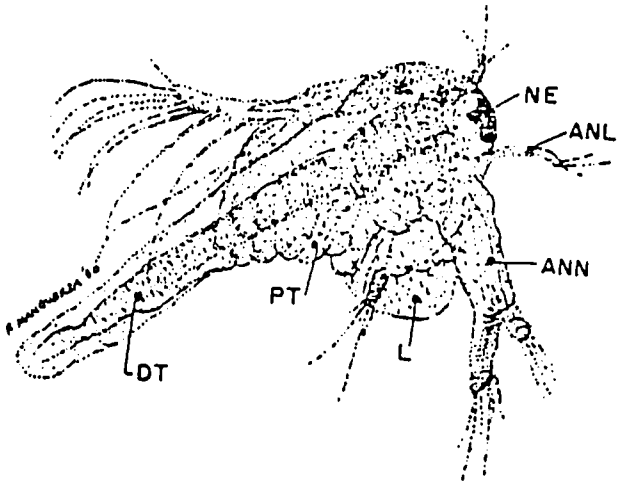


Fig. 8

NE. nauplius eye, ANL. antennula, ANN. antenna, L. labrum, PT. primitive thoracopod, DT. digestive tract.

SUBADULT (20 x)

(male)

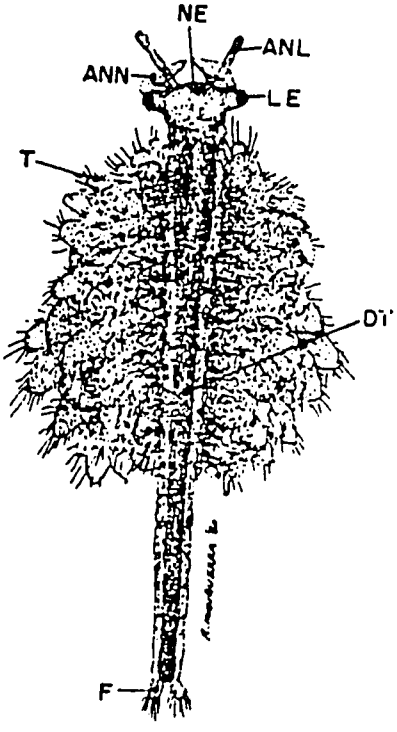


Fig. 9

NE. nauplius eye, ANL. antennula, ANN. antenna developing in hooked

ADULTS (20 x)

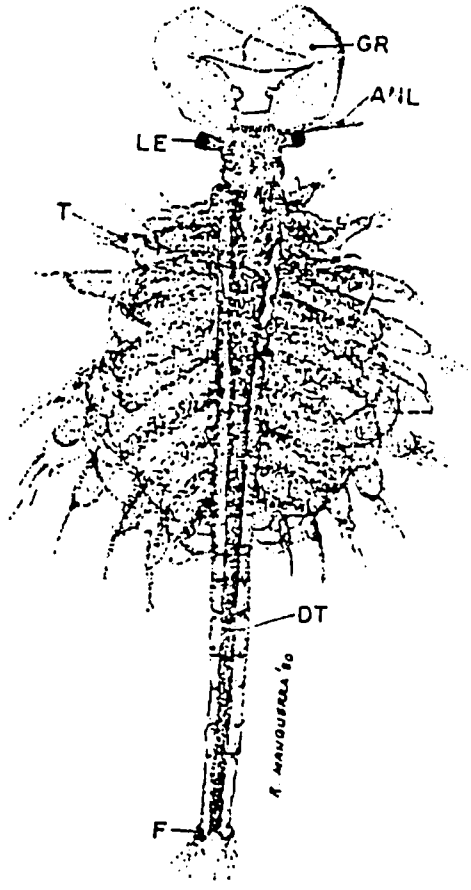


Fig. 10 male

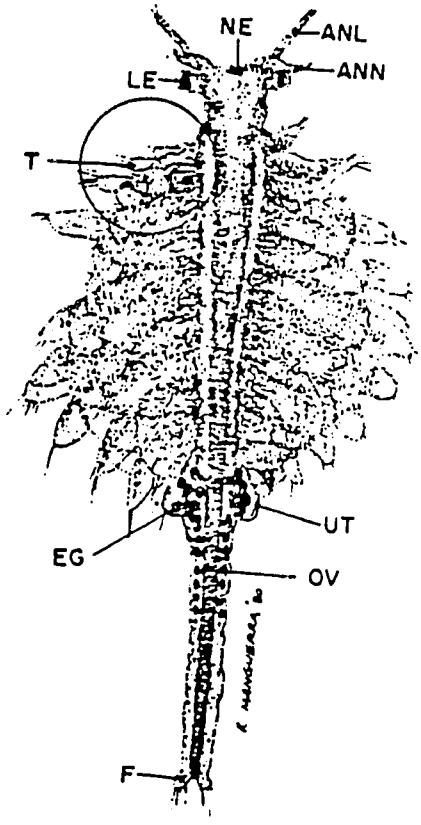


Fig. 11 female

NE. nauplius eye, ANL. antennula, ANN. antenna, LE. lateral eye, T. theracopods, UT. uterus or brood pouch, EG. eggs, OV. ovaries with oocytes, F. furca, DT. digestive tract, GR. graspers (antenna).

DETAIL OF THORACOPODS (40 x)

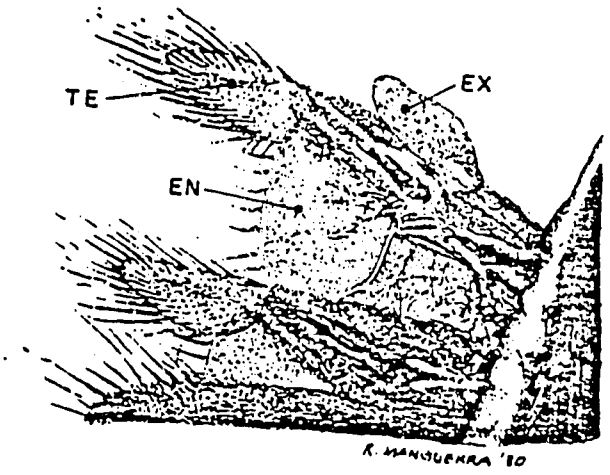


Fig. 12

TE. telepodite, EX. exopodite, EN. endopodite.

RIDING COUPLE (10 x)

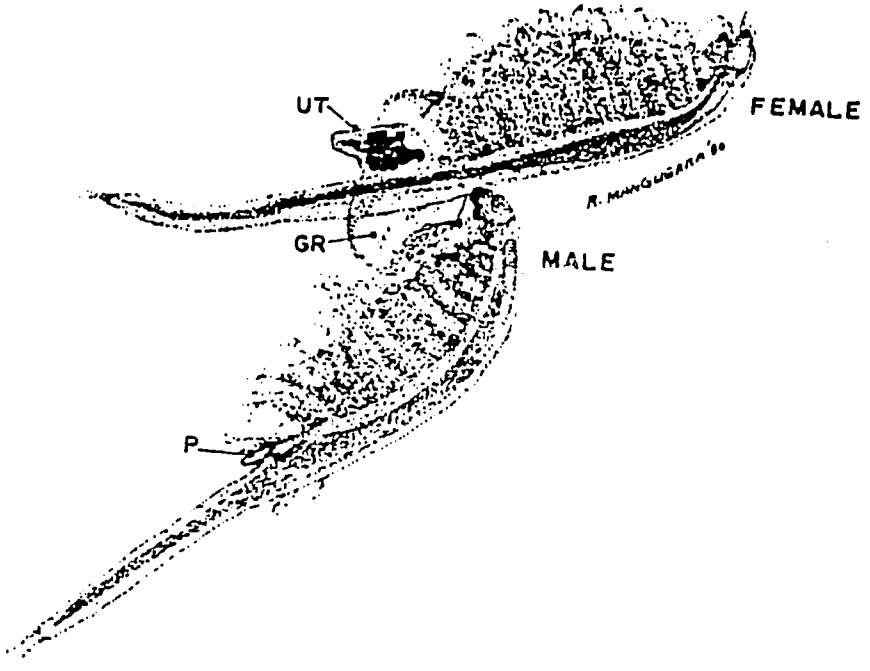
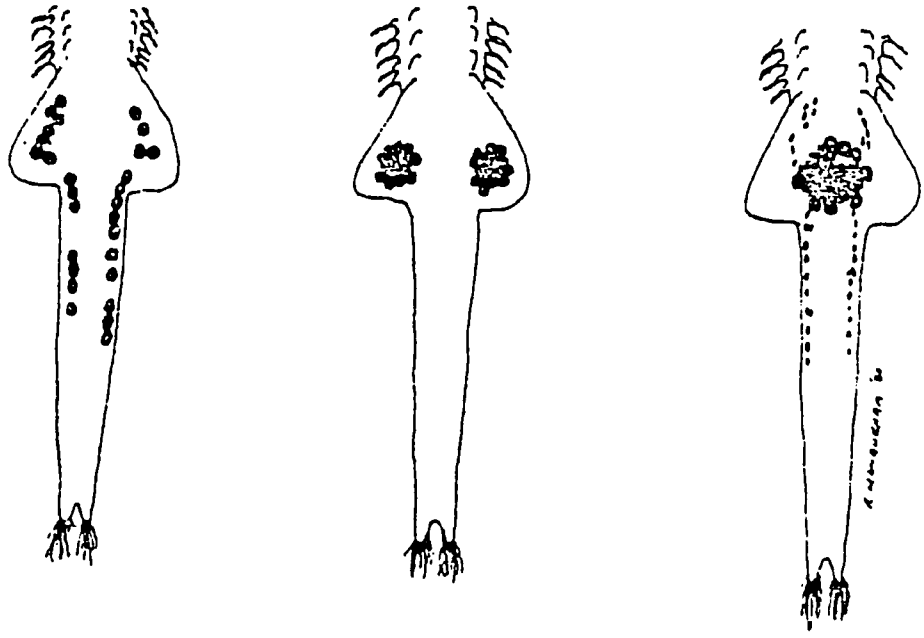


Fig. 13

UT. uterus (or ovisac or brood pouch), GR. male graspers P. penis

EGG DEVELOPMENT (20 x)

Fig. 14



oocytes in ovaries

eggs in oviducts (= lateral pouches of uterus)

eggs in uterus and 2nd brood developing

BROOD POUCH (40 x)

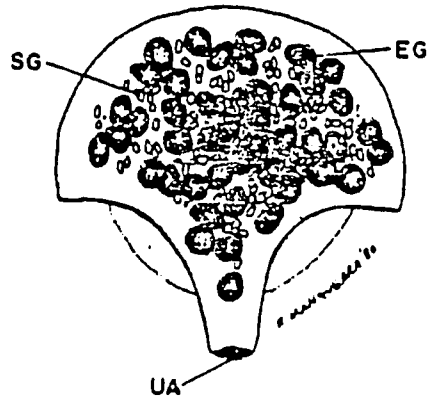


Fig. 15

EG. eggs, SG. shell gland, UA. uterus aperture.

Distinction between oviparous eggs (cysts) and ovoviviparous eggs (will develop into naupliae)

Cysts	"nauplius" eggs
- hard and dark shell	- no shell, soft

PRACTICAL WORK
DETERMINATION OF SALINITY

1. REFRACTOMETER (American Optical)

Scale: 0 - 160 ppt (natural seawater = 32 ppt)

Procedure:
1. Flip back the lid
2. Moisten glass with a few drops of water
3. Replace the lid and hold it with one finger on the label against the glass
4. Hold the instrument against the light and read through the ocular the salinity at the right hand scale (Fig. 16)
5. After use clean with fresh water and dry with tissue or cloth.
6. If salinity exceeds the scale, dilute a sample of the water with freshwater in a measuring cylinder. Mix well, make reading and reconvert result.

2. HYDROMETER (Nektonics, expanded scale)

Scale: 1.000 (0 ppt) - 1.050 (75 ppt)
Specific Gravity (SG)

Procedure:
1. Fill plastic tube with water sample
2. Insert hydrometer. Make sure no airbubbles are attached to hydrometer and that there is no friction between tube wall and hydrometer.
3. After stabilization read lower miniscus down to 0.001 SG accuracy
4. Convert SG - reading to ppt. salinity by using the conversion table. (Table 1)
5. After use, rinse tube and hydrometer with freshwater and dry with cloth.

6. Store with care
7. If salinity exceeds the scale, dilute a sample of the water with freshwater in a measuring cylinder. Mix well, transfer to tube, make reading as above, convert to ppt. salinity and calculate salinity prior to dilution.

exercise: Determine salinity of solution A (about 40 ppt) and B (more than 160 ppt) with both the hydrometer and refractometer.

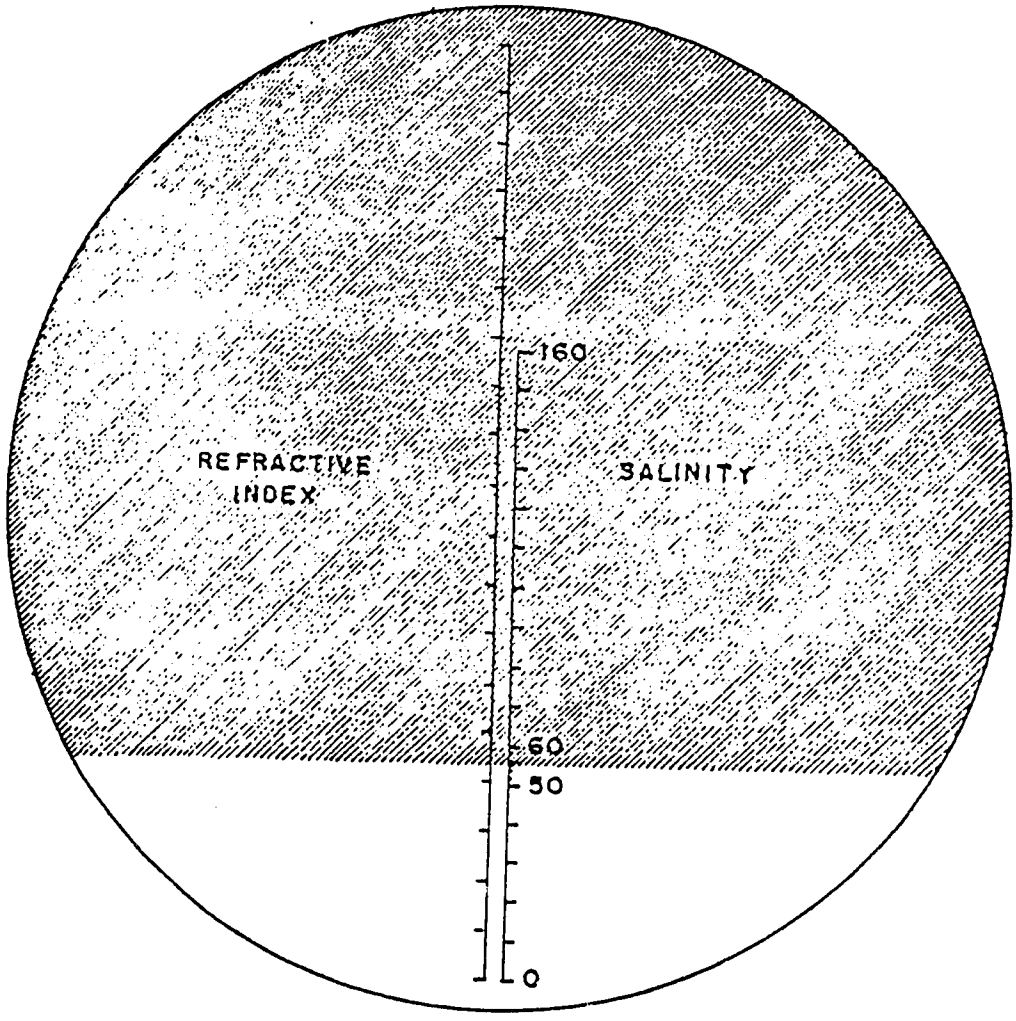


Fig. 16 Refractometer (American Optical)
Reading of 56 ppt. salinity

CONVERSION TABLE

Tested at 26°C or 78°F with A.O. refractometer.

Specific Gravity (S.G.)	Salinity (p.p.t.)
1.000	0
1.003	5
1.007	10
1.010	15
1.013	20
1.016	25
1.019	30
1.023	35
1.026	40
1.029	45
1.032	50
1.036	55
1.040	60
1.043	65
1.047	70
1.049	75
1.053	80

Table I

PRACTICAL WORK
HATCHING ARTEMIA

PROCEDURE AND REQUIREMENTS

1. Maximum hatching density is 7-8 g cysts per liter.
   Use clean natural seawater. If necessary filter the water over a cloth.

2. Aerate hatching container from the bottom with open end airtubes. Never use airstones.

3. Aerate so as not to have sedimentation of the cysts. Therefore use several airlines to obtain strong turbulence and if possible use container with funnel-shaped bottom.

4. Since the cysts need a light impulse during the first hours of hatching, start hatching during daytime, if possible during the morning. Use a transparent container, glass or plastic. If you have to start hatching during the evening, place a TL-light tube in front of hatching container. Cover top of container to prevent insects from entering.

5. Complete hatching takes 24-36 hrs. Take samples now and then to ascertain good hatching.

6. Stand by during hatching in case of power failures or for changing batteries. If nontheless aeration is stopped this will not affect hatching if it happens during the first 10 hrs. Just continue aeration after power has returned.

7. If you hatch for an inoculation, schedule start of hatching in such a way that you can inoculate during late evening or early morning. This is to prevent too high temperatures in the pond upon inoculation.

8. Once hatching is completed, switch off aeration to let empty cyst shells float in layer at surface. (max. 5 minutes). Siphon naupliae from bottom of container. Screen naupliae over 100 µm net and transfer them to a container with clean seawater. Discard hatching water.

9. If required naupliae can be kept alive for max. 1 hour in a volume of seawater twice as much as used for hatching. Keep them in suspension by slow aeration from the bottom of container. (no airstones)

PRACTICAL WORK
INOCULATION

exercise: A pond has a surface of 1400 m^2 and the average depth is 30 cm. An inoculation density of 40 naupliae per liter is required and the hatching efficiency (HE) of the cysts is 4 g/ 1.10^6 naupliae.

1. How many naupliae do you have to inoculate to obtain that density? (Answer: 16,800,000)
2. How many grams of cysts will have to be hatched, if you know that due to manipulation and transport 30% of the hatched naupliae will be lost? (Answer: 96 g).
3. Available hatching containers can hold 8 liters of water. How many containers will be needed and what will then be the hatching density if each container is used at full capacity? (Answer: 2; 6 g cysts per liter)

PRACTICAL WORK
POND MANAGEMENT - WATER INTAKE

WATER INTAKE BY GATE OR CULVERT PIPE

exercise: A pond has a surface of 1500 m^2 and an average depth of 20 cm. Salinity is 150 ppt. You want to lower salinity to 120 ppt by taking in 40 ppt water through a gate or culvert pipe.

How many cm of water should he taken in and what will be the the final total depth? (Answers: 7.5 cm; 27.5 cm)

WATER INTAKE BY PUMP

exercise: A pond has a surface of 2000 m^2 and an average depth of 25 cm. Salinity is 140 ppt. You want to lower salinity to 110 ppt by pumping in water of 40 ppt with a pump of 100 gallons/minute capacity. (1 US gallon = 3.785 liter)

1. What is the volume of water (in m^3) you will have to pump in, and how long will it take? (Answers: 214 m^3; 9.42 hrs.)
2. What will be the final depth after pumping? (Answer: 35.7 cm).
3. Supposing the pump consumes a full tank (4 liters) in 7 hrs. and you start with a full tank, how many extra liters of gasoline should you prepare for? (Answer: 1.4 liter)

PRACTICAL WORK
CYST QUALITY ANALYSIS

1. DETERMINATION OF HATCHING EFFICIENCY (HE)

a. Standard method

Fig. 17 explains the standard procedure to determine the hatching efficiency of a batch of cysts.

equipment needed:
- accurate balance
- 100 ml measuring cylinder
- aerator
- 250 µl automatic pipet with plastic tips or 0.5 ml pipet
- 5 ml plastic tubes with stopper
- washbottle
- rotating hatching apparatus
- Lugol's solution or iodine tincture
- binocular dissecting scope
- plastic petridish for counting

exercise: To determine the hatching efficiency, 5 samples are counted with the standard method, giving the following results:

Tube 1: 110, tube 2: 125, tube 3: 108, tube 4: 112, tube 5: 109.

Calculate:
1. the number of naupliae one gram of product can produce
2. the amount of product needed to produce one million naupliae. (answers: (1) = 180,480 (2) = 5.54 g

b. Simplified method

Fig. 18 explains the simplified method.

equipment needed - accurate balance

- 0.5 ml pipet
- iodine tincture
- binocular dissecting scope
- plastic petridish for counting

DETERMINATION OF HATCHING PERCENTAGE (HP)

Fig. 19 explains the method.

equipment needed:
- 100 ml measuring cylinder
- aerator
- iodine tincture
- plastic petridish for counting
- binocular dissecting scope

exercise: In 5 samples from an Artemia hatching, the counted numbers of naupliae and cysts + shells are:

sample	naupliae	cysts + shells
1	82	130
2	93	125
3	74	110
4	96	124
5	88	112

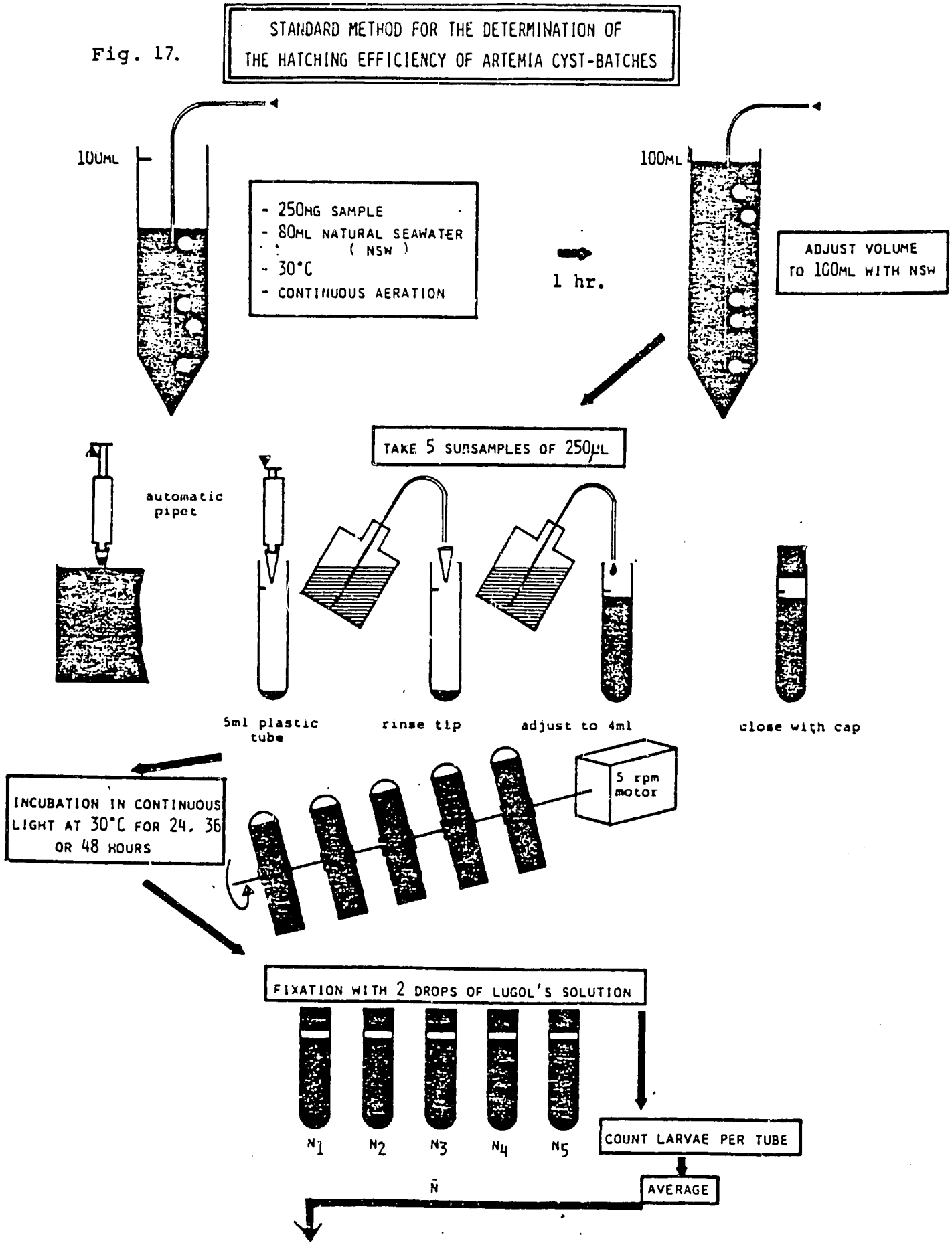
What is the average hatching percentage (HP) for that batch of cysts ? (answer: HP = 72%)

exercise: Of a certain batch of cysts the HE = 4.5 g/ 1.10^6 naupliae and HP = 85%.

1. How many grams of the product do you have to hatch to obtain 250,000 naupliae? (Answer: 1.13 g)
2. How many cysts are there in that of amount of product? (Answer: 294,118)
3. Supposing that 5% of the weight of the product is debris, what is the weight of 1 cyst in µg? (Answer: 3.65 µg).

from: Artemia Reference Center

Fig. 17.

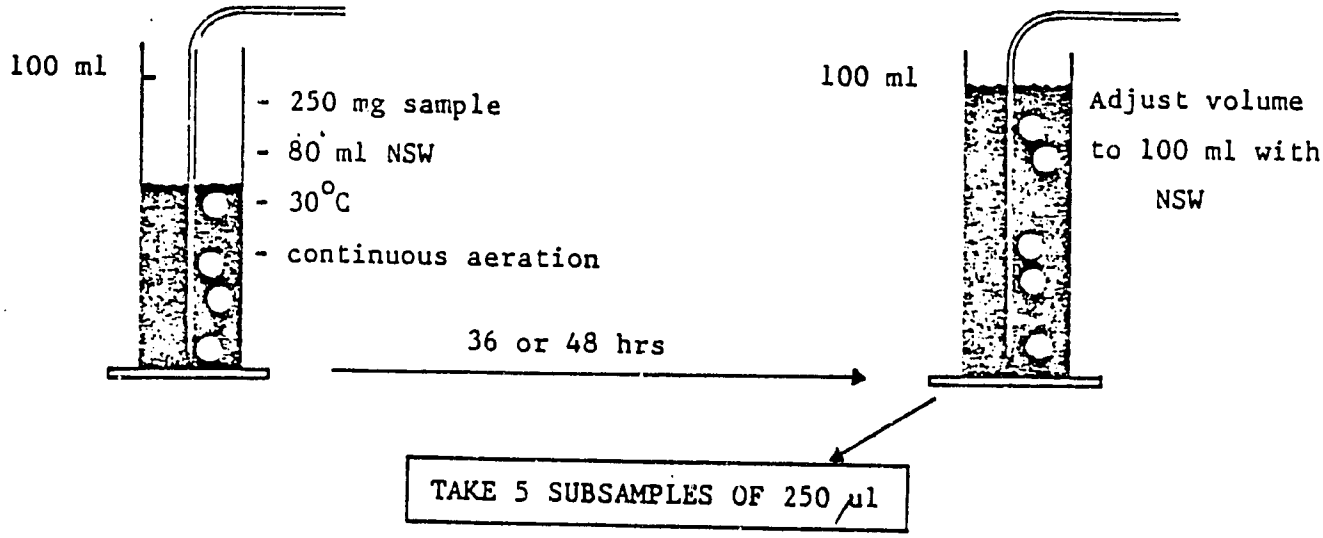


HATCHING EFFICIENCY (HE) or

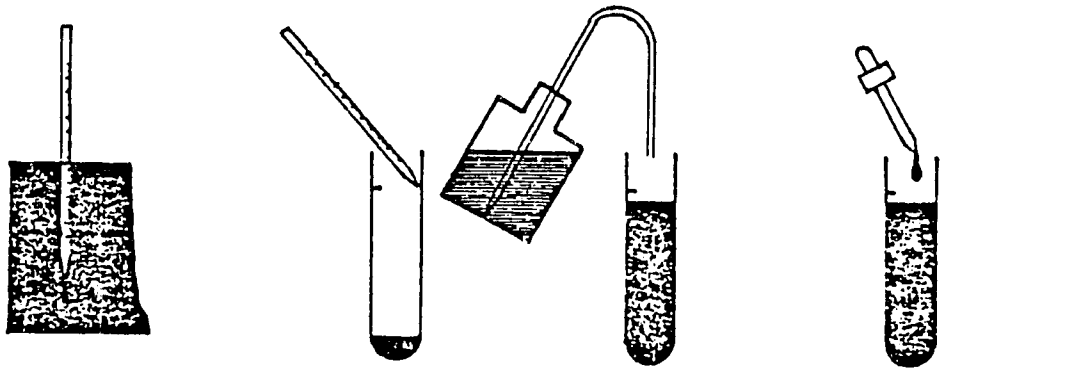
$$\frac{\text{number of larvae}}{\text{one gram product}} = \bar{N} \times 4 \times 100 \times 4 \quad (1)$$

Fig. 18

SIMPLIFIED METHOD FOR THE DETERMINATION OF THE HATCHING EFFICIENCY



TAKE 5 SUBSAMPLES OF 250 µl



COUNT LARVAE PER VIAL

AVERAGE $\bar{N}$

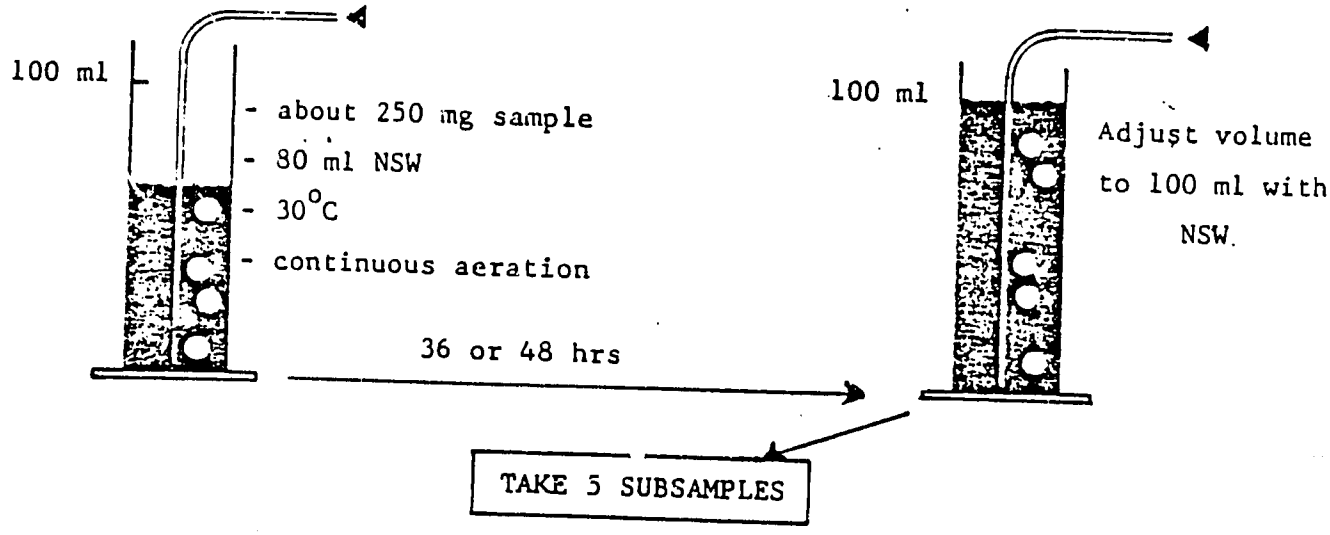
HATCHING EFFICIENCY (HE)

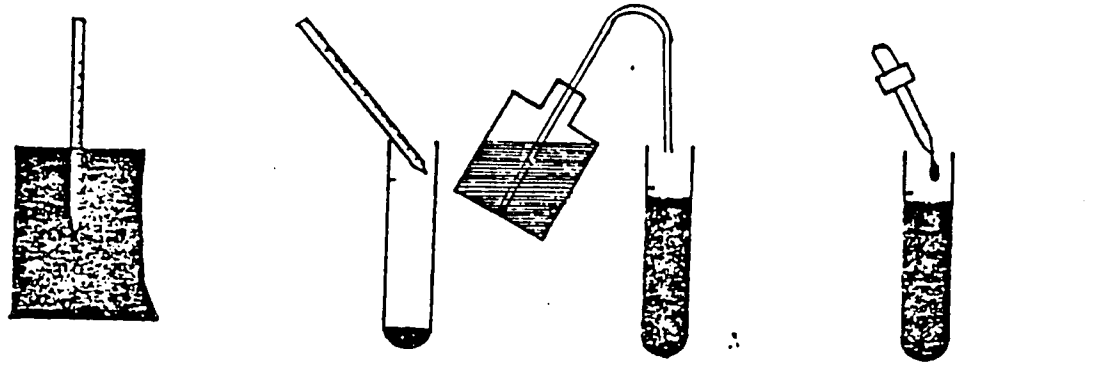
$$\frac{\text{number of larvae}}{\text{one gram product}} = \bar{N} \times 4 \times 100 \times 4 \quad (1)$$

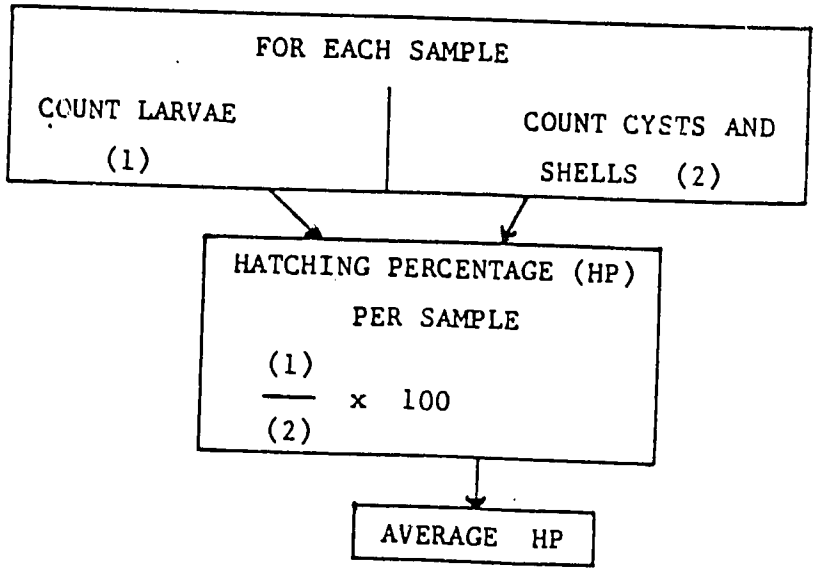
or

$$\frac{\text{weight of product}}{\text{one million naupliae}} = \frac{1{,}000{,}000}{(1)}$$

Fig. 19 METHOD FOR THE DETERMINATION OF THE HATCHING PERCENTAGE







PRACTICAL WORK
PROCESSING AND STORAGE OF ARTEMIA CYSTS

CLEANING

1. Harvest cysts from pond. Drain excess water over 100 or 200 µm screen and transport drained cysts to processing site.
2. Wash cysts over 400 and 100 µm screen with freshwater. Large dirt particles, dead Artemia, algae debris etc. will be retained on 400 µm screen and cysts with finer debris on 100 µm screen.
3. Transfer the cyst mass to a brine container for preliminary cleaning and temporary storage until further processing. Mix now and then by aeration or manually by means of a stick. The brine will dehydrate the cysts and a first part of the heavy debris will sink to the bottom. Full cysts and light debris will float in a layer at the surface.
4. Transfer the cyst mass to a funnel-shaped brine container. Aerate at half height of container. This separates the rest of the heavy debris from the cysts. This brine separation must be carried out for 24 hrs. to guatantee complete dehydration (dented cysts). With the funnel-shaped container, the floating cyst mass can be very precisely separated from the sedimented debris.
5. Wash the cysts thoroughly with freshwater over a 100 µm screen to remove all salt. This step should not take longer than 5 minutes.
6. Transfer the cysts to a funnel-shaped freshwater container. Again agitate the surface by means of slow aeration from half the height of the container. Full cysts will sink and light debris and empty cysts will float. This freshwater separation should not take longer than 15 minutes. Otherwise the cysts will reach the hydration level which sets off metabolism. Collect full cysts.
7. Wash shortly over 100 µm screenbag with freshwater spray.
8. Remove excess water by shaking out water, gently squeezing the bag and dipping dry with absorbent cloth.

DRYING

1. Distribute the cysts in thin layers on a drying surface, consisting of a table or trays made of wire screen covered with cotton muslin (tight woven grade). This will absorb water. By shaking a woven wire basket (5 mm mesh) containing the cysts, the cysts will be sifted through the mesh and be evenly distributed over the drying surface.

2. Place these trays in a drying box or on outdoor racks for sun-drying. The latter will not affect viability of the cysts if they were well dehydrated during the brine separation (step 4). The drying box can be made of ordinary wood. Make slits at the bottom and top of the box for air exchange and use a 75 Watt light bulb as heating element, fixed on the bottom of the box.

3. Stop the drying process as soon as the cysts do not lose further weight. (completed dehydration).

4. Pulverize the cyst lumps and screen over a 300 to 500 µm screen to powder the cysts.

STORING

Depending on expected duration of storage three possibilities exist.

1. For storage up to a few months, cysts can be stored in vials containing clear brine. This can already be done after brine separation in the cleaning phase (step 4), thus omitting the air-drying procedure.

2. If storage is for a term up to 6 months or a year, it is sufficient to store the air-dried cysts in closed glass or plastic vials filled to the brim. As long as they are kept dry viability will not be affected significantly over a period of 1 year. There is no need to keep them in the refrigerator.

3. If storage is for more than a year, or if the cysts have to be packed for commercial purposes, it is necessary to pack them dry and under vacuum or nitrogen atmosphere (away from oxygen). Commercial brands mostly vacuum can their cysts.

APPENDIX

RELATED LITERATURE

DE LOS SANTOS, C.S., Jr.; P. SORGELOOS. E. LAVINA; A. BERNARDINO (1979) Successful inoculation of Artemia and production of cysts in manmade salterns in the Philippines. International Symposium on the brine shrimp, Artemia salina (Corpus Christi-Texas-USA,, Aug. 20-23, 1979), in press.

SORGELOOS, P. (1978) The culture and use of the brine shrimp, Artemia salina, as food for hatchery-raised larval prawns, shrimps and fish in Southeast Asia. FAO Workpaper THA;75:008/78/WP/3, pp. 50.

SORGELOOS, P. (1979) List of commercial harvesters-distributors of Artemia cysts of different geographical origin. Aquaculture. 16. 87-88.

TUNSUTAPANICH, A. (1979) Cyst production of Artemia salina in salt ponds in Thailand. FAO Workpaper THA:75:008/80/WP/9, pp. 14

VOS, J. & TUNSUTAPANICH, A. (1979) Detailed report on Artemia cyst inoculation in Bangpakóng, Chachoengsao Province. FAO Field Document THA/75/008, pp. 54.

VOS, J. (1979) Brine shrimp (Artemia salina) inoculation in Tropical Salt Ponds: A preliminary Guide for Use in Thailand. FAO Workpaper THA:75:008/79/WP/4, pp.14.