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नं.१४१, मार्शल्स रोड, एगमोर, मद्रास – ६०० ००८.

भाकृअनुप
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(Indian Council of Agricultural Research)
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Cover Photo: Adult tiger prawns (*Penaeus monodon)*.

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PREFACE

Shrimp farming is developing at a rapid pace. About 85,000 ha area is currently under the traditional and the new systems, and the total area is expected to reach the 100,000 ha target fixed for year 2000. The demand for shrimp seed, particularly of the tiger shrimp Penaeus monodon, is very high and there is an increasing gap between supply and demand, which should be bridged in time if the potential is to be realised. The exploitation of wild seed resources from the estuarine region has been so rampant as to cause deep concern for conservation of the biodiversity and the resources.

A large number of shrimp hatcheries have been constructed, and the number is on the increase, but these are not without their share of problems. One of the major constraints is the lack of sufficient broodstock which acts as the limiting factor in hatchery seed production. The present overriding reference of the industry is on the tiger shrimp, ignoring all other valuable species, and this has only compounded the problem.

The Central Institute of Brackishwater Aquaculture, since its inception in 1987, has been seized of the problems of shrimp seed production and has carried out research on broodstock development and induced maturation of P. monodon and P. indicus. Based on our experience, it could be stated that P. indicus responds far more easily and favourably than P. monodon under captive conditions in broodstock facility.

*Knowledge base and technical skill are the two important requirements for successful operation of the hatcheries. Scientific and technical information needs to be disseminated on a wider basis to make it available to the industry. The Bulletin on "***Development of broodstock and maturation of tiger prawn,** *Penaeus monodon* **in captivity***" has been prepared as part of the Institute's information dissemination efforts. Although maturation is presented briefly, it contains all relevant information, including inventory requirements and approximate costs. I would like to express my appreciation to Dr. L. Hanumantha Rao, Shri. M. Kathirvel, and Dr. P. Ravichandran Scientists of the Institute who prepared this Bulletin and to Shri K. N. Krishnamurthy, Principal Scientist for its editing.*

It is our endeavour to reach the farmers and industry engaged in brackishwater aquaculture with information and technologies developed at the Institute to help assist sustainable development of this sector which is one of the vital arms of fisheries development in the country. It is hoped that this Bulletin will be found useful by the industry.

Madras - 8 — **K. ALAGARSWAMI**
2.1.1995 — DIRECTOR

CONTENTS

1. INTRODUCTION

The tiger prawn, *Penaeus monodon* is the most preferred species for shrimp farming. The availability of quality seed is a prerequisite for the rapid expansion of prawn culture in our country. At present the total estimated area under shrimp cultivation is about 28,000 ha under extensive and improved extensive system, about 2000 ha under semi-intensive and intensive system and 50,000 ha under the traditional practices. It is expected that by 2000 AD, about 25,000 ha under improved extensive, 20,000 ha under semi-intensive and 5000 ha under intensive systems would be brought under shrimp farming. The estimated seed requirements to meet the demand is around 19 billion. Availability of seed from nature is seasonal, location specific and of a mixed quality. Hence, to ensure a steady supply of pure quality seed in large quantities, hatchery production under controlled conditions is the only alternative.

Shrimp hatchery operations rely on the availability of spawners from the sea, which is seasonal and limited. In order to meet the increasing and growing demand for seed, it is essential to develop techniques for maintaining a captive broodstock and induce them to mature under controlled conditions, for sustained production of quality seed.

As a part of Research and Development activity, the Central Institute of Brackishwater Aquaculture has undertaken research on controlled reproduction of *P.monodon* and a technology package for broodstock development and induced maturation of *P.monodon* in captivity is presented in this Bulletin.

2. REPRODUCTIVE BIOLOGY OF *P. monodon*

P. monodon females attain larger size than males and sexes can be distinguished based on morphological features.

2.1. Reproductive system

The male reproductive system consists of a pair of testes, *vas deferens* and terminal ampoules which open at the base of fifth pair of walking legs. The endopodites of the first pair of swimming legs are united to form a tubular structure known as petasma (Plate 1 A). The petasma helps in the transfer of spermatophores into the thelycum of females.

The female reproductive system comprises of a pair of ovaries and oviducts. The oviducts terminate into gonopores at the base of third pair of walking legs. Females possess an oval-shaped structure called thelycum, situated in the space between the last three pairs of walking legs (Plate 1 C). The thelycum of *P. monodon* is of closed type and hence the deposition of spermatophores during mating occurs only when the female is in newly moult condition. The spermatophores in the thelycum will be lost with the exuvia when the prawn moults.

2.2. Ovarian maturation stages

On the basis of external examination of ovaries, the following stages of maturation are recognised in *P. monodon:*

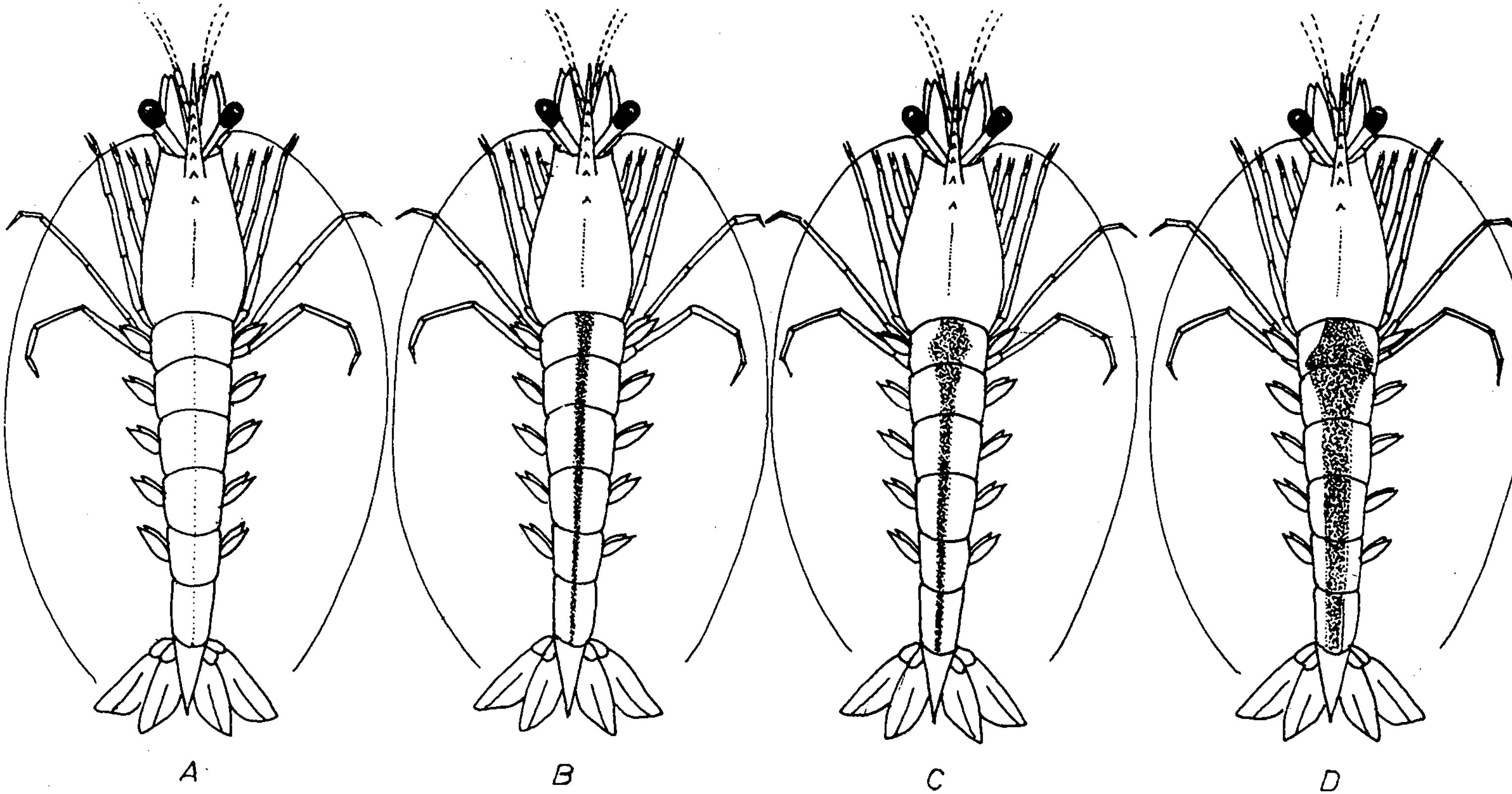

Fig. 1. Maturation Stages : A. Immature/ spent; B. Early maturing; C. Late maturing; D. Mature.

Stage I (Immature) (Fig.1 A)	:	Ovaries thin, transparent, not visible to the naked eye through exoskeleton.
Stage II (Early maturing) (Fig.1 B)	:	Ovaries thin, increase in size in the anterior and middle lobes, visible as thin linear band through exoskeleton.
Stage III (Late maturing) (Fig.1C)	:	Ovaries visible to the naked eye through the exoskeleton as thick dark linear band, expand at the first abdominal segment.
Stage IV (Mature) (Fig.1 D)	:	Ovaries dark green in colour, assume a distinct diamond shape clearly visible at the first abdominal segment.
Stage V (Spent) (Fig.1 A)	:	Ovaries appear as a thin linear band as in the case of Stage I, partially spent ovaries having either the anterior or posterior lobes.

3. BROODSTOCK DEVELOPMENT

The source of broodstock of *P. monodon* is either from wild or from ponds.

3.1. Broodstock from wild

Adult *P. monodon* females weighing above 90 g and males above 60 g are collected either from the sea or from backwaters. Females collected from the sea are always preferred since they respond to induced maturation faster and produce relatively higher percentage of quality eggs.

3.2. Broodstock from ponds

In pond grown specimens, prawns of above 8 months old are suitable for maturation. Development of broodstock of *P. monodon* in grow out ponds is done by two ways. *P. monodon* postlarvae-20 are reared at low stocking density (1/m^2) in well prepared earthen ponds of 0.05 - 0.10 ha with water depth of 1 m for a period of 8-10 months, using natural food and supplemented with pellet feeds @ 5 - 10 % of the total biomass. Water management includes 10 - 30 % exchange of seawater everyday. Rearing of postlarvae can also be done in two phases. In the first phase, postlarvae (PL - 20 stage) are reared upto 30 - 40 g at a density of 3/m^2 over a period of 5 months. In the second phase, 40 g prawns are reared upto 60 - 85 g at a density of 1/m^2 over a period of 5 months, using fresh feeds such as clam or squid meat @ 10% of the total biomass distributed 2 times in a day and supplemented with pellet feeds given @ 3 - 5 % of the total weight of prawns once in the evening. Water management includes 10 - 30 % exchange of seawater per day.

Salinity at which the prawns are grown has a strong influence on their response to induced maturation. Prawns collected from low saline areas (less than 30 ppt) have to be maintained at sea water salinity of 33 - 36 ppt in broodstock holding tanks for a period of one month using fresh and pellet feeds and changing 100% seawater everyday, before ablating them.

3.3. Transportation and prophylactic treatment

The prawns collected from wild or from ponds should be transported with minimum stress. For short-term transportation of less than 2 hours, open containers could be used. 20 - 30 prawns can be easily transported in 200 liter tank without any mortality. For transportation involving longer duration, the animals have to be packed under oxygen in polythene bags @ 1 prawn / 5 l of water and transported in thermocol lined boxes at reduced temperatures.

Gradual acclimation to sea salinity is essential for prawns collected from low saline waters. The prawns are disinfected in 25 - 50 ppm formalin for 1 hour before transferring them into the broodstock holding tanks.

4. INDUCED MATURATION

P. monodon males weighing above 40 g are found to be mature in estuarine water (Plate 1 B: arrows indicate the presence of spermatophores), while females do not mature in captivity or in brackishwater. In nature, females move to the sea for attaining maturity. The female maturity is governed by a set of neurohormones secreted by X organ situated in the eyestalk and thoracic ganglion. The gonad inhibiting hormone (GIH) is secreted by the X organ and the gonad stimulating hormone is secreted by the thoracic ganglion. The GIH is reported to be more active in estuarine conditions and in captivity, thereby preventing maturation. Removal of one eyestalk helps to reduce GIH level and accelerates ovarian development (ovulation).

4.1. Selection of females

After acclimatisation to the seawater conditions, healthy females without any sign of disease or injury, having spermatophores in the thelycum should be selected from the adults maintained in the holding tanks for induction of maturity. The spermatophores in the thelycum could be easily seen by the vertical, milky white streaks on both the sides of the thelycum (Plate 1 D, E & F). The moulting stage of the prawn is one of the criteria for selection of females. Eyestalk ablation during newly moult condition will lead to mortality due to additional stress. In pre-moult or ready to moult prawns, ablation results in immediate moulting which will delay the process of maturation. Hence, only hard shelled intermoult prawns should be selected for ablation.

4.2 Eyestalk ablation

Unilateral eyestalk ablation is done by any one of the following methods:

a) Cutting
b) Incision - pinching and
c) Electrocauterisation.

Cutting is done by complete removal of eye with eyestalk using a sharp instrument. Incision-pinching is done by making an incision on the eye ball using a sharp blade and squeezing out the contents of the eyestalk. Electrocauterisation is done by cutting the eyestalk using cautery apparatus.

Of the three methods, the use of electrocautery has the advantage of causing minimum stress, less loss of body fluids and healing of wound.

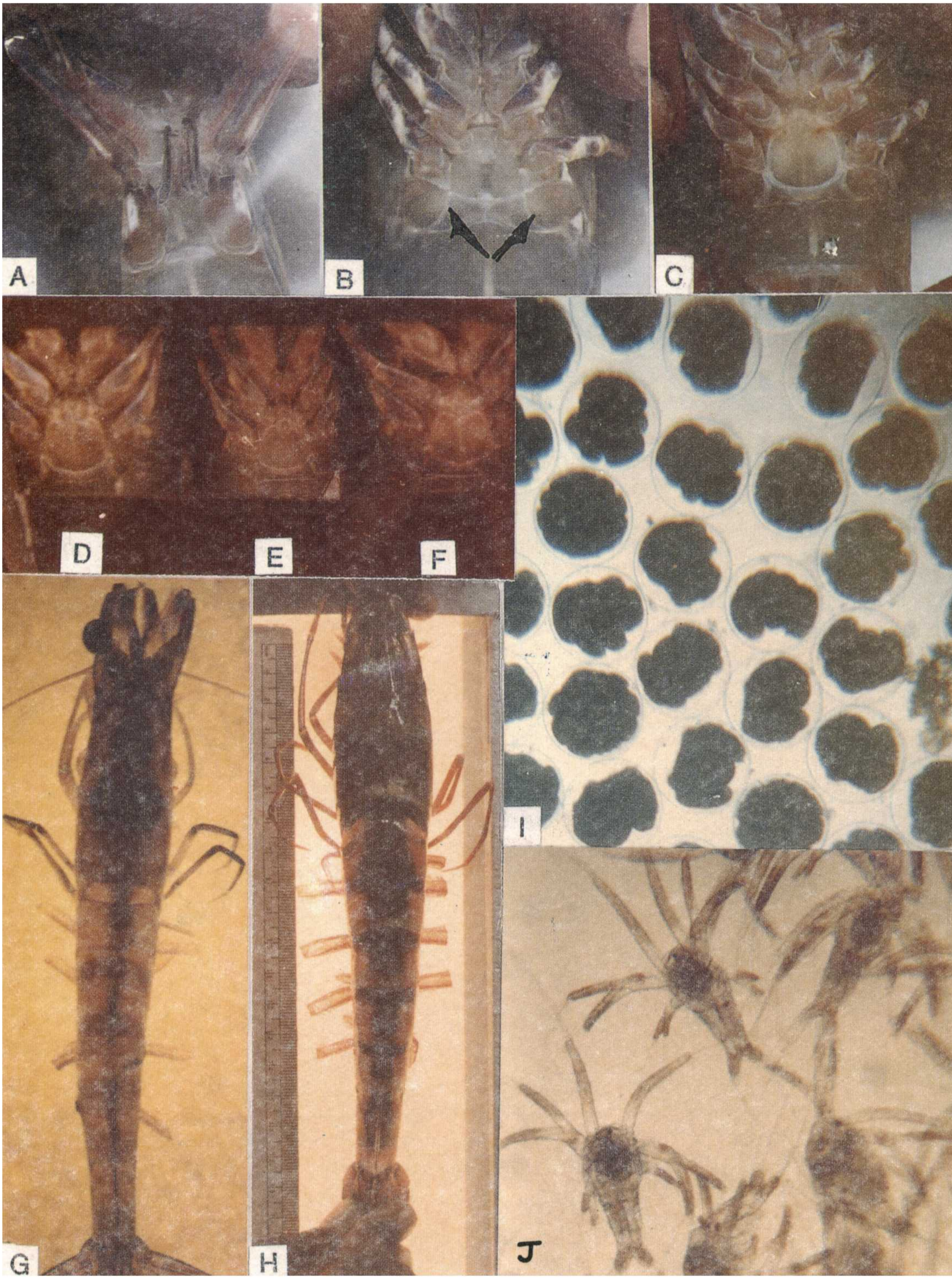

PLATE 1. A. Petasma; B. White mass seen at the base of the fifth walking legs of matured male; C. Thelycum; D & F. Thelycum with spermatophores; E. Thelycum without spermatophores; G. Ablated female after 4 days; H. Ablated female with mature ovary; I. Developing eggs; J. Healthy nauplii.

Ablation should be done as quickly as possible to minimise stress. Soon after ablation, the females should be released immediately into the maturation tanks.

4.3. Stocking

The ablated prawns are stocked in the maturation tanks along with unablated males @ 4 numbers/m^2 with water depth of 80 - 100 cm. Stocking of females and males in the ratio of 2 females : 1 male ensures best mating success.

4.4. Physico-Chemical quality requirements

The physical and chemical quality of seawater and light intensity in the maturation tanks has strong influence on the maturation process. Optimum environmental conditions required for maturation in captive females of *P. monodon* are given in Table 1.

TABLE 1. Optimum conditions for *P. monodon* maturation in captivity

Housing	:	Ventilated, roofed shed
Tank size	:	12 - 15 t capacity. circular or rectangular made of fibre glass (FRP) or concrete
Light intensity	:	Reduced, 100 lux (artificial) dim light
Light quality	:	Blue or green
Photoperiod	:	12 hour light : 12 hour dark
Water depth	:	80 - 100 cm
Water Quality		
Salinity (ppt)	:	30 - 36
Temperature (^oC)	:	28 - 30
pH	:	8.0 - 8.5
Dissolved oxygen	:	Saturation by continuous aeration
Stocking rate	:	4 Numbers/m^2
Stocking size	:	Females 90 - 150 g; Males 60 - 80 g
Sex ratio	:	2 females : 1 male
Water management	:	100 % exchange/day using filters or 200%exchange by flow through system per dav

Feeds

Fresh		Clam, mussel, squid, crab & oyster meat @ 15% of the total biomass per day, Polychaete worms @ 6% of the total weight or *Artemia* biomass @ 3% of the total biomass
Artificial (Pellet)	:	2% of the total biomass/day
Feeding schedule	:	Four times in a day

4.5. Seawater quality

The seawater obtained either from the sea or from a borewell sunk in the intertidal zone to a depth of 3 m, is pumped into reservoirs where it is treated with Calcium hypochlorite or Sodium hypochlorite depending on the turbidity, vigorously aerated to expel chlorine and allowed to settle for 6 - 12 hours. The residual chlorine is neutralised with required quantities of sodium thiosulphate and passed through gravitational sand filters or pressure sand filters. EDTA is added @ 10 ppm as a chelating agent. The treated seawater is used after passing through 10 micron fabric filters for broodstock and maturation, and through 5 micron cartridge filters for spawning and hatching.

4.6. Feeds and feeding

It is essential to feed the ablated prawns with quality feeds, since the fecundity and quality of eggs depend on the quality of feed given. Both fresh and pellet feeds are used as maturation diets (Table 1).

4.6.1. Fresh feeds

Fresh feeds such as clam (*Meritrix casta*), mussel (*Perna viridis*), squid (*Loligo* sp.), crab (*Scylla* spp. & *Charybdis* sp.), having similar amino acid profile as prawns, polychaete worms (*Glycera* sp.) and *Artemia* biomass rich in long- chain polyunsaturated fatty acids are used as maturation diets. Clam, squid, mussel or crab is given on rotation @ 15% of the total biomass distributed 4 times in a day, while polychaete worms (6% of the biomass) or *Artemia* biomass (3% of the biomass) are given once in a day.

4.6.2. Pellet feeds

Research on nutritional requirement of penaeid prawns showed that polyunsaturated fatty acids (PUFA) such as arachidonic acid, eicosopentaenoic acid and docosohexaenoic acid are essential for the prawns to attain maturity. Therefore, pellet feeds with 50% protein and 10% PUFA are to be provided for achieving desirable results. In recent years, commercial dry pellet feed specially prepared for feeding broodstock are available. The best results can be achieved by using fresh feeds along with pellet feeds.

4.7. Monitoring of spawners

The progress of ovarian development in the ablated prawns is monitored 3 days after ablation by using an underwater light. The ablated prawns start maturing 4 days after ablation (Plate 1 G). The females in late maturing (III stage) and mature (IV stage)(Plate 1 H) stages are transferred to the individual spawning tanks after prophylactic treatment with 50 ppm formalin for one hour. The ablated prawns mature and spawn repeatedly 3 - 5 times during the intermoult period and remain productive for 50 - 60 days.

The health of the broodstock prawns is also checked regularly and periodical treatment with formaliln @ 50 ppm for 30 minutes is given.

4.8. Spawning, fertilization and hatching

The mature prawns are introduced @ 1 prawn per spawning tank (500 l capacity) filled with filtered seawater and provided gentle aeration. Spawning takes place usually in the early morning hours. The eggs are released from the gonopores, simultaneously a portion of the contents of spermatophores is ejected out of the thelycum, thus paving the way for an external fertilisation. The spent spawners are returned to the maturation tank. The eggs are siphoned out, cleaned with seawater to remove scum and transferred to 4 l capacity basin. After thorough mixing, two 10 ml samples are taken and the number of eggs present are counted physically, which is raised to the total volume of water to obtain the total number of eggs released. About 100 eggs are examined under a microscope to determine the quality of eggs.

Wild females have a fecundity of 2,00,000 to 10,00,000 eggs depending on the size of the prawn, with an average of 4,00,000 eggs/female. But ablated females of 90 - 150 g spawn 1,00,000 to 5,00,000 eggs with an average of 2,00,000 eggs/prawn. The fecundity and the quality of eggs gradually decreases with repeated spawnings.

The eggs from each spawning are transferred into 300 l tanks containing filtered seawater and provided gentle aeration. Those eggs (Plate 1 I) having the normal pattern of development of embryo hatch out into nauplii (Plate 1 J) in 15 - 17 hours after spawning, depending on the water temperature. To estimate the nauplii production, four 100 ml random samples are counted visually and multiplied the number to the total volume of water.

5. INFRASTRUCTURE FACILITY

The infrastructure facility required for a production of 100 million nauplii per annum from captive broodstock of *P. monodon* is given below.

(A) Sheds (AC sheets)

1.	Maturation shed	140 m^2
2.	Blower/generator/pump house	50 m^2
3.	Laboratory/dormitory	60 m^2

(B) Tanks (Cement)

1. Maturation tanks - 12 t capacity — 6 No
2. Water storage tanks - 50 t capacity — 2 No
3. Over head tank - 20 t capacity — 1 No
4. Broodstock holding tanks - 20 t capacity — 2 No
5. Earthen ponds - 0.05 ha — 2 No

(C) Tanks (Fibre glass- FRP)

1. Spawning tanks 500 l capacity (round) — 12 No
2. Hatching tanks 300 l capacity (round) — 6 No

(D) Essential equipment

1. Air blowers (Roots) 5 - 10 HP, Pressure 0.2 - 0.3 kg/m^2 volume 4 - 5 liters/m^2/minute — 2 No
2. Generator 30 KVA — 1 No
3. Pumps (Centrifugal and electrically operated) 5 HP — 2 No; 3 HP — 2 No
4. Pressure sand filters (flow rate 20 t/hour) — 2 No
5. Salinity Refractometer 0 - 100 ppt — 1 No
6. pH meter — 1 No
7. Heating elements with thermostat control 1 KVA — 12 No
8. Microscope — 1 No
9. Deep freezer 350 l — 1 No
10. Cartridige filters 5 micron — 2 No
11. Thermometer 0 - 50°C — 2 No
12. Balance to weigh upto 500 g — 2 No
13. Electrocautery apparatus — 1 No

(E) Systems

1. Seawater intake system
2. Aeration system
3. Electrification

The lay-out of tiger prawn broodstock facility is presented in Fig.2.

6. COST AND RETURNS ANALYSIS

The approximate cost to be incurred for the fixed assets and recurring expenditure is presented below:

	Rs. in lakhs
A. **Fixed costs**	
Ponds / Tanks	6.40
Sheds	5.00
Equipment	11.00
Total	22.40
B. **Operational cost**	
Broodstock	1.20
Feed	0.80
Chemicals	1.50
Power	1.80
Consumables	0.30
Manpower	1.62
Total	7.72
C. **Income**	
Sale price of 100 million nauplii @ Rs.20,000/million	20.00
D. **Gross profit** (C - B)	12.28
E. **Net profit** (after allowing 20% interest on capital cost and 20% depreciation on equipment cost)	6.10

7. ACKNOWLEDGEMENTS

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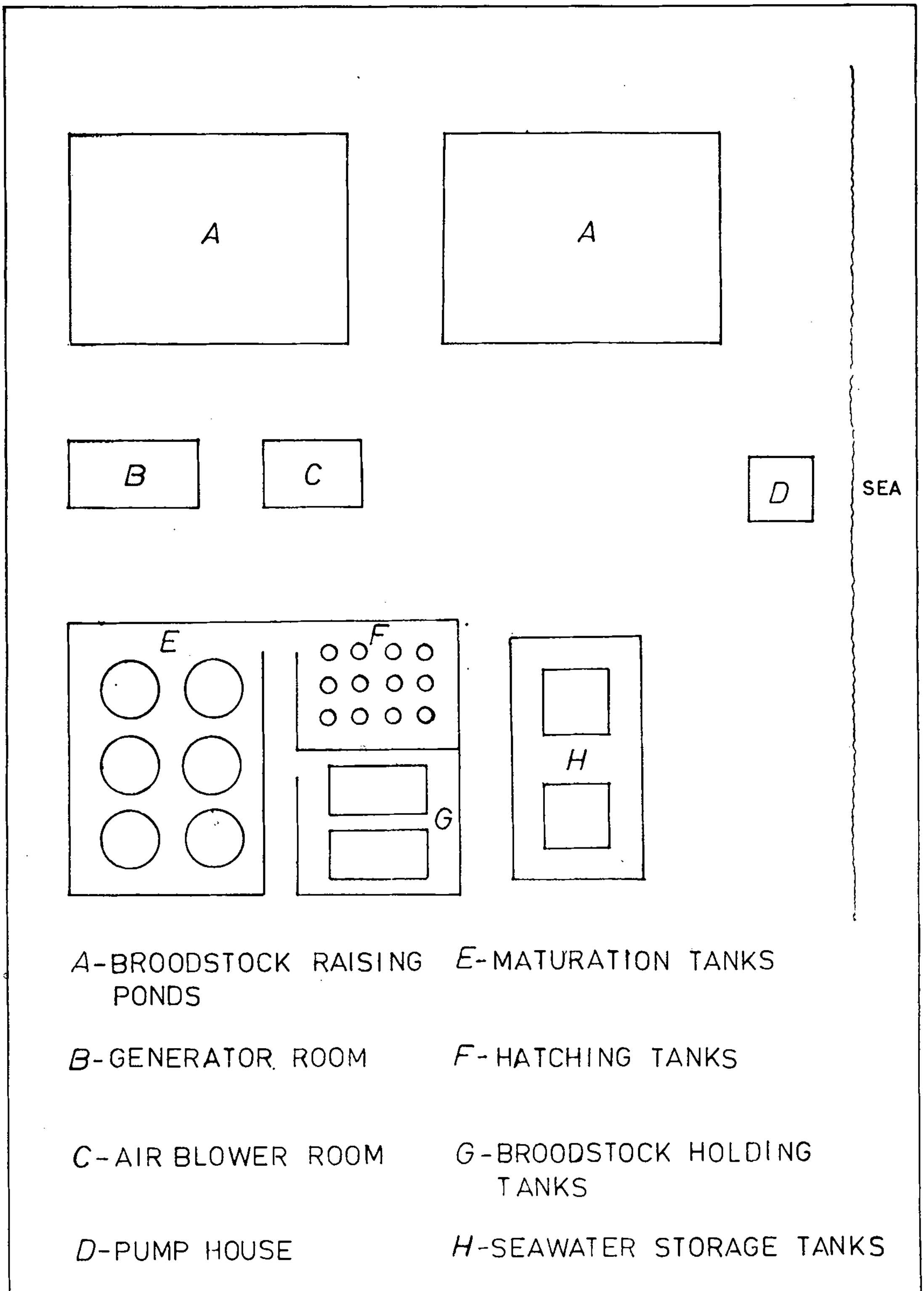

Fig. 2. Lay-out of tiger prawn broodstock facility.