A SIMPLIFIED HATCHERY TECHNIQUE FOR MASS PRODUCTION OF PENAEID PRAWN SEED USING FORMULA FEED

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ABSTRACT

A technique for large-scale production of penaeid prawn seed using a dry microparticulate formula feed, NPCL-17, is described. The details of preparing the feed which is compounded from inexpensive, locally available raw materials such as groundnut oil cake, fish meal, dried mantis shrimp, prawn waste and tapioca and fortified with vitamins and minerals are given. The larvae are grown in outdoor tanks filled with 2 m^3 of seawater, filtered through 60-micron mesh nylobolt cloth. The daily ration of formula feed is offered in 4 equal instalments at 6 hourly intervals and the larval tanks are vigorously aerated. Apart from providing nutrition for the larvae, the feed, under the influence of sunlight, helps to create a natural ecosystem conducive for their survival. The easy-to-dispense dry feed has greatly simplified the larval rearing procedures and has given an average survival rate of 66.7% from nauplius to postlarva. The rationale of the feeding schedules and the particle size of the feed offered are discussed in the light of the changes taking place in the functional morphology of the feeding appendages during larval development.

INTRODUCTION

In the context of increasing global demand for prawns and the stagnation of their production from the existing capture fisheries, most of the developing countries in the tropical region have given high priority for promoting prawn culture in impounded coastal waters and are engaged in setting up hatcheries to provide the prawn culturists with adequate supply of prawn seed. Ever since Hudinaga (1942) succeeded in rearing penaeid prawn larvae under laboratory conditions, several workers have evolved more sophisticated procedures to increase the survival of the larvae in culture systems (See Muthu 1982). It is well recognised that the key factor in successful rearing of penaeid larvae is the provision of appropriate feed for the larvae at the various stages of development (Cook and Murphy 1966; 1969; Aquacop 1977; Mock et al 1980). The expensive procedures involving high skill and technology needed for developing and maintaining cultures of suitable live-feed organisms such as diatoms, brine shrimp larvae, rotifers, cladocerans, nematodes, etc., have been the major constraints in large-scale production of prawn seed for aquaculture purpose.

Recently several substitute inert feed stuffs and feed formulations have been tried to overcome this difficulty. In small-scale rearing experiments Hirata et al (1975) used powdered soycake to rear the larvae of *P. japonicus*, while Villegas and Kanazawa (1980) were successful with a particulate compounded diet. Shigueno (1975) reported that powdered formula feed could be used with fair results in rearing the larvae of *P. japonicus*. Jones et al (1979) reared the larvae of the same species to PL_1 stage on microparticulate and microencapsulated diets. Hameed Ali (1980) and Alikunhi et al (1982) used blended crustacean tissue suspensions prepared out of *Mesopodopsis* sp., *Metapenaeus dobsoni* and *Squilla* sp. to rear the penaeid larvae on a large scale.

Realising the importance of large-scale hatchery production of prawn seed for coastal aquaculture the Central Marine Fisheries Research Institute, Cochin, initiated work on different aspects of spawning and rearing of penaeid prawns under controlled conditions at their Narakkal Prawn Culture Laboratory (NPCL). Among the various research projects undertaken at this laboratory with the objective of simplifying the larval rearing procedures for mass production of prawn seeds, special attention was given to development of microparticulate larval feeds using locally available inexpensive raw materials. The results of this study concerning the larval rearing of the Indian white prawn *Penaeus indicus* H. Milne Edwards are reported here.

Material and Methods

The equipment and infrastructural facilities available at the NPCL were fully utilised for conducting these experiments. Besides standard laboratory equipment, the facilities included oil-free compressed air grid, filtered-seawater supply system, mass cultures of phytoplankton and other live feed organisms, portable plastic pools, brood-stock maintenance facility, growout ponds of varying sizes and glasshouse. The seawater used in the laboratory was pumped from the sea into large indoor tanks where it was allowed to settle for two days and then filtered through 60-micron nylobolt cloth. Even after filteration this seawater usually contained diatoms to the extent of 600-2000 cells/ml. The larvae used for these experiments were obtained from the routine prawn-breeding programme of NPCL using farm-grown spawners of *P. indicus*, induced to mature in the broodstock facility.

Preparation of formula feed NPCL-17

Locally available inexpensive raw materials such as prawn waste (a waste product of the prawn-processing industry consisting of prawn heads, appendages and peeled cuticle), mantis shrimp (a usually discarded by-catch of the trawlers), groundnut oil cake, fish meal and tapioca were used as main ingredients. The above materials were dried and powdered separately in a micropulveriser, fitted with 150-micron sieve, to form a fine powder. A 'feed base' was first prepared by thoroughly mixing these powders in the following composition.

Dried prawn waste	..	25.0%
Dried whole mantis shrimp	..	25.0%
Groundnut oil cake	..	37.5%
Fish meal	..	12.5%

Dried tapioca, powdered in the same micropulveriser, served as binder and carbohydrate source. For every 100 g of the feed base 20 g of tapioca powder was used. The feed was fortified with vitamins and minerals. To every 100 g of the feed base the following were added. Vitamin A (as acetate) USP 2000 I.U., thiamine mononitrate I.P. 5 mg, riboflavin I.P. 1 mg, nicotinamide I.P. 5 mg, pyridoxin hydrochloride I.P. 1.5 mg, calcium pantothenate USP 5 mg, cyanocobalamine I.P. 0.5 mg, ascorbic acid I.P. 25 mg, calciferol I.P. 200 I.U., DL-alpha tocopherol acetate 0.75 mg, biotin 0.025 mg, magnesium phosphate (dibasic) 24 mg, manganese hypophosphite 0.3 mg, dried ferrous sulphate 5.3 mg, calcium phosphate 0.104 g, calcium lactate 0.2 g, potassium dihydrogen phosphate 0.1 g.

The ingredients were mixed together in the proportion given above and blended with 40% by weight of water to obtain moist granules. They were kept in trays and steamed in a cooker for 10-15 minutes. After cooking, the cooked material was made into a dough, extruded through a pelletiser using a 3-mm die and dried in a hot-air oven at 65-70°C for 12 h. The dried pellets were powdered in micropulveriser using a 50-micron sieve and stored in air-tight polyethylene containers at room temperature.

The proximate composition of the formula feed NPCL-17 is as follows:

Proteins	..	36.8%
Lipids	..	10.1%
Carbohydrates	..	29.8%
Ash	..	18.9%
Moisture	..	4.4%

Laboratory experiments in glass beakers

Initially, indoor laboratory experiments were carried out in glass beakers in order to determine whether *P. indicus* larvae could be reared through the various stages of devlopment exclusively on a formulated diet. One litre of specially filtered (20 micron filter) seawater of 35 ppt salinity was taken in each beaker and 50 numbers of P_7 obtained from the same brood were introduced. Continuous aeration was provided in each beaker through air stones and the experiment was carried out in ambient room temperature, which varied from 29.0 to 30.5°C. Three different feeding schedules were adopted in the beakers and the treatments were replicated thrice in a randomised block design. Feeding of the larvae was carried out once a day at the rates as follows:

Treatment	Feed	Feeding rate
1	No feed	
2	Mixed diatom culture dominated by *Chaetoceros affinis*	80-100 x 10^3 cells/ml
3	Formula feed NPCL-17	0.16 mg/larva/day

The daily ration of formula feed given in Treatment 3 was identical with the quantity of feed used in similar experiments with *P. japonicus* by Hirata et al (1975) and Villegas and Kanazawa (1980). The rearing medium was changed every day, when all the larvae were counted and staged under a binocular microscope. Since all the larvae did not metamorphose into the next stage at the same time, the stage of development of the larvae (S) in each beaker was expressed as

$$S = \frac{D}{\text{Total number of larvae in beaker}}$$

Where D = Σ Number of larvae in each stage times arbitrary value of that stage. For this an arbitrary value was assigned to each stage of larvae as follows:

Stages	*Arbitrary value*
Nauplius (N)	0
Protozea-1 (P_1)	1
Protozea-2 (P_2)	2
Protozea-3 (P_3)	3
Mysis-1 (M_1)	4
Mysis-2 (M_2)	5
Mysis-3 (M_3)	6
Postlarva-1 (PL_1)	7

Larval rearing in outdoor pools using formula feed NPCL-17

Fifteen experiments were carried out in 2 m^3 capacity circular plastic-lined pools kept inside a glass house in bright sunlight (50000 to 120000 lux). The pools were disinfected, cleaned and filled with seawater on the day the prawns were expected to spawn. The water in the pools was vigorously aerated. The hatched-out nauplii were stocked in the rearing pools at stocking densities varying from 6 to 52 $N_1/1$ of seawater. Although the nauplius is a non-feeding stage 2-3 g/m^3 of formula feed were added to the pool on the day of stocking itself. The dosage of formula feed was increased to 4 g/m^3/day during the protozoeal stage and to 5 g/m^3/day in the mysis stage. The daily dose of feed was divided into four equal portions and added to the pool at 6 hourly intervals. By the time the larvae reached P_1 stage, the phytoplankton originally present in

the seawater medium, increased to 17000 to 42000 cells/ml. When the sunlight was intense the phytoplankton concentration often reached 80000 to 100000 cells/ml and under such circumstances the dosage of formula feed was reduced. It would appear that the unutlized formula feed in the medium was broken down by bacteria, thereby providing nutrients for development of phytoplankton under the influence of sunlight.

The larvae were examined under the microscope in the morning and evening every day to see if they were feeding well. From P_2 stage onwards 1/3 volume of water from the pool was siphoned out through a filter box (300 micron mesh) and replaced with fresh seawater. Vigorous aeration was maintained throughout the period of rearing to promote aerobic decomposition of the sediments. The experiments were carried out in ambient temperatures varying from 25.4 to 34.6°C.

RESULTS

Laboratory experiments in glass beakers

The survival and development of the larvae in the beaker experiments are shown in Fig. 1 and Fig. 2 respectively. For each treatment the values plotted represent the averages of the three replicates. In the beakers where no feed was given all the larvae died after the second day in P_1 stage indicating that the larvae, having exhausted the yolk reserves in nauplius stage, required energy from external sources of food at this stage.

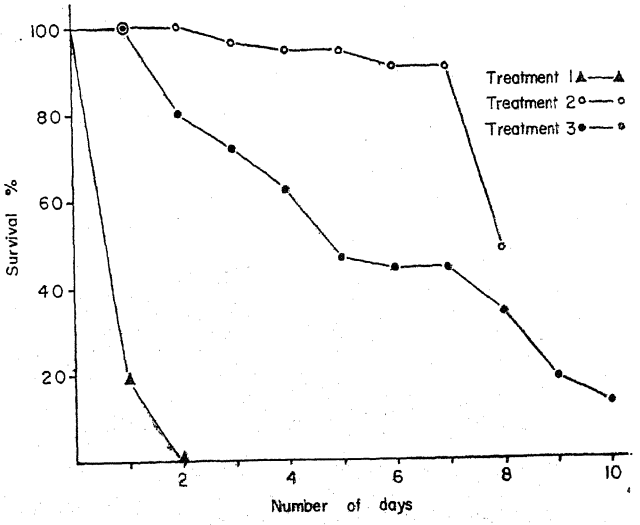

FIG. 1. Survival of *P. indicus* larvae on different feed treatments in 1-l beakers.

In phytoplankton-fed cultures (Treatment 2), however, the development of the larvae followed a normal sequence and the survival was as high as 90% up to M_3 stage, which they attained on the 7th day. It is obvious that up to this stage the feed provided was nutritionally adequate for their normal growth and survival. The survival rate, however, declined sharply to 48% between M_3 and PL_1, indicating that the phytoplankton alone was not sufficient beyond M_3 stage.

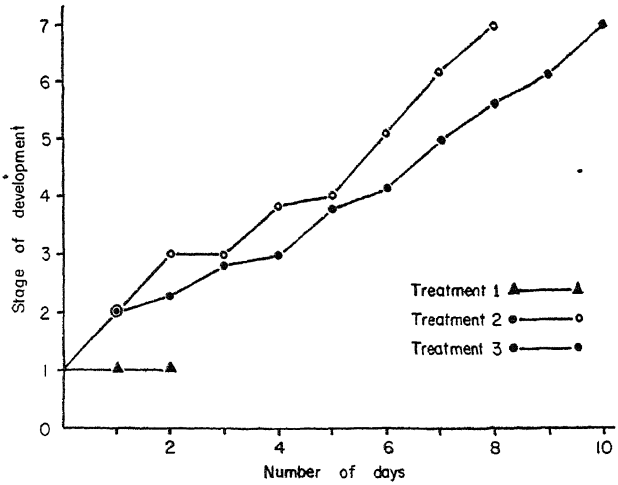

FIG. 2. Development of *P. indicus* larvae during different feed treatments in 1-1 beakers.

In Treatment 3, using formula feed, mortality at each larval stage was significant and the development was delayed, the PL_1 stage having been attained in 10 days, i.e, two days later than in Treatment 2. Besides, the survival rate of 12.5% at PL_1 stage was also relatively low indicating the formula feed by itself is not a complete food for the larvae. Nevertheless, the fact that 12.5% larvae developed and survived up to the PL_1 stage on this diet alone showed that they were able to utilise this formula feed to a limited extent for their survival. The experiments in large outdoor pools were planned on the basis of evaluation of these results.

Larval rearing in outdoor pools using formula feed NPCL-17

These experiments were conducted with the objective of utilising the formula feed in combination with the phytoplankton production naturally taking

place in seawater in the presence of sunlight. The 15 sets of experiments making use of 921000 N_1 to produce 614000 PL_1 of *P. indicus* gave an average survival rate of 66.7%. The survival rate in individual experiments, however, ranged from 32.2% to 96.8% (Table 1). The development period from N_1 to PL_1 ranged from 7 to 8 days in the warmer months and 9 to 10 days in cooler months.

When the recorded survival rates are plotted against stocking densities (Fig. 3) there seems to be no correlation between them. At the highest stocking density tried during the present experiment (52 N_1/l) the survival rate was 92.3% whereas at the lowest stocking density (6 N_1/l) the survival rate was 66.7%. The lowest survival rate of 30.2% was obtained in the experiment in which stocking density was 26.5 N_1/l. The usual inverse relationship between stocking density and survival rate was not evident at the stocking densities tried. This may become relevant at higher stocking densities. In other words the system can sustain stocking densities higher than 52 N_1/l.

It is interesting to note that the quantity of formula feed added to the pools, when calculated in terms of the amount of feed per larva per day, did not show any relationship to the survival rate of the larvae at the level of population densities employed (Table 1). Therefore, it was obvious that the larvae had also made use of other food from the medium (i.e., the phytoplankton which developed naturally in the pools) in addition to the formula feed provided. Periodic microscopic examination of the larvae showed that their gut contents consisted chiefly of greenish substance particularly in the early protozeal stages and, in addition, the formula feed was also seen in gut of the larvae from the P_2 stage onwards. That the larvae have been ingesting the formula feed was evident from the long fecal trails they developed 10 minutes after introduction of the feed and the good survival of the larvae was clear indication of its assimilation by the larvae. Since the pools were set up outdoors exposed to sunlight

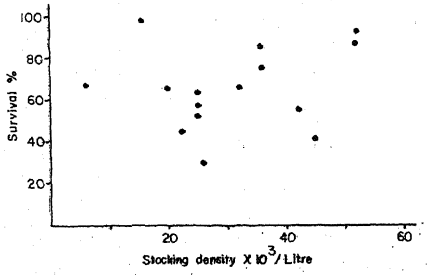

FIG. 3. Survival rate of *P. indicus* larve at different stocking densities.

and since the medium was not sterlized the normal primary and secondary production process continued to take place in the medium, thereby providing a natural ecosystem for the prawn larvae.

Discussion

The experiments conducted during this study have given an insight into the role played by microparticulate feeds in larval rearing of penaeid prawns. The exclusive use of formula feed NPCL-17 in the beaker experiments using specially filtered seawater resulted in poor survival and delayed development of the larvae, indicating that the feed by itself is not adequate for the larvae. But the same feed when used in the large pools exposed to sunlight gave high survival rates (average 66.7%) and the duration of larval development was normal. In the latter case, the phytoplankton which was obsereved to develop in the larval rearing pools under the influence of sunlight has been an additional source of nourishment to the larvae. It was possible that the unutilised particles of the formula feed might have also acted as organic fertilizer stimulating the growth of the phytoplankton. It is interesting to note that Hirata et al (1975), Hameed Ali (1980) and Alikunhi et al (1982), who reared penaeid larvae on particulate diets in outdoor tanks exposed to sunlight, have reported the presence of good diatom concentrations in their larval rearing containers. The success of their rearing methods is possibly due to the additional nutrition provided to the larvae by the diatoms growing in the medium. The point to be emphasised here is that the particulate diets and tissue suspensions may not be effective as larval feed when they are grown indoors under poor light conditions.

Rearing of penaeid larvae in outdoor tanks in which natural growth of phytoplankton is stimulated by adding chemical nutrients is, no doubt, closely similar to the technique of rearing them in outdoor tanks with particulate feed. But the former method is however impracticable for large-scale commercial rearing in hatcheries due to the uncertainty in development of phytoplankton in the rearing media. Regulating the growth of phytoplankton consisting of desired species at optimum concentrations in larval culture media has been found to be very difficult under conditions obtaining in hatcheries. It is in this context that the success of larval rearing using formulated dry particulate feeds assumes importance.

The size of the food particles offered to the larvae seems to have profound influence on the success of rearing penaeid larvae. NPCL-17 was dispensed in the form of micropulverised powder obtained after passing through a seive of 50-micron mesh. The particle size of the conventional species of diatoms offered as natural feed of penaeid larvae fall within 5 to 20 microns. *Chaetoceros affinis* which was the dominant diatom in the seawater at Narakkal was 5-8 microns in size. Jones et al (1979) found that the average size of particles ingested by the larvae of *P. japonicus* increased from about 10 microns in P_1 to

TABLE 1. *Mass rearing of* P. indicus *larva in outdoor pools using formula feed.*

Experiment	Dates	Duration in days	Temprature range °C	Stocking density $N/_1$	N_1	Population Nos. x 10^3 P_1	M_1	PL_1	Quantity of feed added mg/larva/day	Percent survival rate N_1 to PL_1
1	4-3-82 to 12-3-82	8	30.0-32.5	35.5	71	66	64	60	0.07-0.14	84.5
2	,,	8	30.0-32.5	42.5	85	73	52	48	0.06-0.13	56.5
3	,,	8	29.5-32.1	22.5	45	35	27	20	0.06-0.13	44.4
4	,,	8	29.0-32.0	32.0	64	49	47	42	0.08-0.14	65.6
5	21-3-82 to 29-3-82	8	31.0-33.0	6.0	12	9	8	8	0.42-0.67	66.7
6	26-3-82 to 3-4-82	8	31.0-34.0	20.0	40	39	30	26	0.13-0.25	65.0
7	29-4-82 to 6-5-82	7	32.0-34.6	25.0	50	41	35	26	0.12-0.20	52.0
8	27-11-82 to 5-12-82	8	28.0-31.5	26.5	53	38	28	16	0.13-0.19	30.2
9	1-12-82 to 10-12-82	9	27.3-29.8	36.0	72	63	58	54	0.11-0.12	75.0
10	4-12-82 to 13-12-82	9	27.4-31.4	45.0	90	53	41	37	0.07-0.09	41.1

11	5-12-82 to 14-12-82	9	27.2-29.9	15.5	31	30	30	30	0.19-0.26	96.8
12	10-12-82 to 19-12-82	9	26.7-31.4	25.0	50	43	31	28	0.06-0.12	56.0
13	5-1-83 to 14-1-83	9	25.4-28.9	25.0	50	41	38	32	0.04-0.14	64.0
14	21-1-83 to 31-1-83	10	26.0-29.1	52.0	104	101	98	96	0.06-0.12	92.3
15	21-1-83 to 31-1-83	10	26.0-29.1	52.0	104	100	95	91	0.06-0.12	87.5

Container 2 ton plastic pool; Salinity range 33.00-34.95 ppt; pH range 7.82-8.57

about 28 microns in M_3 stage. While rearing the larvae with particulate diets the frequency of feeding is to be given due consideration, as excess of left-over food in the medium causes pollution of water resulting in mortality. Delayed feeding also produces disastrous effects. These problems can be overcome by continuous aeration of the medium and by adding the daily ration in instalments at periodic intervals. Accordinly, in the present experiments, the daily ration was divided into 4 equal parts and dispensed at 6-hourly intervals. Hirata et al (1975), Jones et al (1979 and Villegas and Kanazawa (1980) have given the daily ration of particulate feed in a single dose. Hameed Ali (1980) and Alikunhi et al (1982) have dispensed the daily ration of wet tissue suspension in 5-h intervals.

Very high survival rates up to M_3 stage have been recorded in the indoor experiments conducted in 1-m^3 capacity pools on an exclusive diet of mixed phytoplankton without using any zooplanktonic elemets. This confirms the observations of Muthu (1982), who showed that the feeding appendages of protozoea and mysis of penaeids are adapted only for filter feeding. In these stages the numerous long setae and the close-set setules of the second maxilla form an effective filtering device for seiving out the phytoplankton cells from the sea water. The mandibles are weak and the first three pereopods have only rudimentary chelae which are incapable of grasping moving prey. When M_3 metamorphoses into PL_1, the maxillary filter is abruptly lost, the serrated teeth on the mandible are replaced by a sharp cutting edge and the chelae on the first three pereopods become functional. The postlarva ceases to be a filter feeder and becomes capable of capturing and dealing with larger food organisms thereby transforming into an effective carnivore. That there is a sudden change in the feeding habits when M_3 metamorphoses into PL_1 is also confirmed by the experiments of Jones et al (1979), who demonstrated through accumulative particle size analysis of microcapsules in the medium, that the mean particle diameter of the microcapsules increased after the larvae had fed on them during the protozoea and mysis stages, while it abruptly declined during the postlarva stage. The sudden decline in survival rate from M_3 to PL_1 observed in our beaker experiments in which the larvae were fed only with mixed phytoplankton cultures (Fig. 1) also support the view that the feeding habits change abruptly at the time of metamorphosis. Emmerson (1980), using pure cultures of *Thalassiosira weissflogii* as feed, has shown that the filtration rate of the larvae of *P. indicus* increased steadily from 2500 cells/larva/h in P_1 stage to 12,000 cells/larva/h in M_3 stage and then suddenly declined to 6,000 cells/larva/h in early PL_1 stage. He also attributed the reduced filtration rate of the early PL_1 stage to the reduction in the size of the filter-feeding appendages at this stage. In the light of these observations the conventional practice of feeding zooplanktonic organisms to the prawn larvae during the mysis stage should be critically re-examined. Since the larvae at this stage are not physically equipped to capture moving prey organisms, large quantities of the costly *Artemia* nauplii that are

given as feed for the early mysis stage larvae may be wasted, although those accidentally coming into contact with the larvae may be retained by the coarser setae on the maxillipedes and eaten. It is therefore suggested that the introduction of zooplanktonic feed in larval tanks can be delayed till the M_3 stage is about to metamorphose into PL_1. At the NPCL, however, the postlarvae are fed on coarser particles of the formula feed passed through a 300-micron mesh seive.

In comparision with the methods of rearing penaeid larvae extant in various countries the new method using dry formulated feed has the following advantages:

1. Eleborate arrangements for developing and maintaining liv-feed cultures are not required. This will result in considerable savings in space, labour and equipment, thereby reducing the cost of production of prawn seed.

2. Rearing procedures are greatly simplified so that even unskilled labourers with a little training can manage the hatchery operations.

3. Formula feed is prepared out of inexpensive raw materials through simple processes requiring no specialised equipment.

4. The feed can be prepared in large quantities in advance and stored in airtight containers to be used off the shelf whenever necessary, whereas the commonly used live feeds and wet tissue suspensions will have to be freshly cultured or processed each time.

5. The dry particulate feed can also be dispensed through automated feeding devices in sophisticated hatchery systems.

ACKNOWLEDGEMENTS

The authors are gratefully indebted to Dr. E. G. Silas, Director, Central Marine Fisheries Research Institute, for his constant help and guidance during the course of this work and to their colleagues at the NPCL for their co-operation and assistance.

REFERENCES

ALIKUNHI, K. H., G. MOHAN KUMAR, S. RAVINDRAN NAIR, K. S. JOSEPH, K. HAMEED ALI, M. K. PAVITHRAN AND P. K. SUKUMARAN. 1980. Observations on mass rearing of penaeid and *Macrobrachium* larvae, at the Regional Shrimp Hatchery, Azhikode, during 1979 and 1980. *Bull. Dept. Fish, Kerala*, 2(1): 68 pp.

AQUACOP, 1977. Elevage larvaire de penaeides en milieu tropical. Groupe de travail acquaculture, ICES, 10-13 Mai, Brest, France. *Actes de colloques du CNEXO*, 4: 179-191.

COOK, H. L. AND M. A. MURPHY. 1966. Rearing penaeid shrimp from eggs to postlarvae. *Proc. 12th Ann. Conf. S.E. Ass. Game & Fish Comm.*, 19: 283-288.

COOK, H. L. AND M. A. MURPHY,. 1969. The culture of larval penaeid shrimp. *Trans. Am. Fish. Soc.*, 98(4): 751-754.