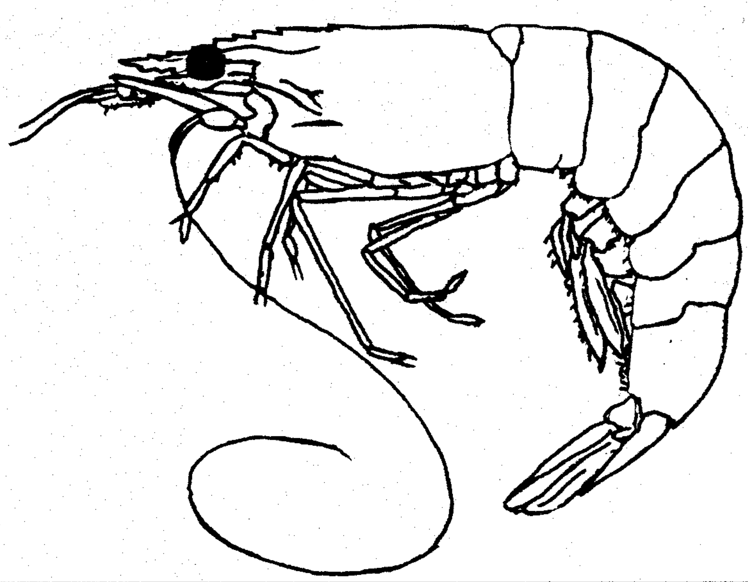
Final Report to the Overseas Development Administration NRED Research Programme for the period April 1988 - March 1991

STUDIES ON THE CULTURE OF TROPICAL PRAWNS (PENAEIDS) WITH PARTICULAR REFERENCE TO HUSBANDRY, DISEASE AND NUTRITION.

Research Project R4443 NRG 522/832/8A



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Ap A.5. Higuera-Ciapara I., Inglis V. and Brown J.H., 1991. Antibiotic residue detection in shrimp and salmon muscle using the Charm-7000 system. Paper to be presented at the WAS Conference in Puerto Rico, June 1991. A5

Ap A.6.a. Wood J.F. and Brown J.H., 1990. Report on a visit to India. "The potential pond productivity of the tiger shrimp (*Penaeus monodon*) from semi-intensive artisanal culture in India. Report to ODA as part of the Bay of Bengal Post-harvest fisheries project. R 1607 (R). A6a

Ap A.6.b. Wood J.F. and Brown J.H., 1990. Making high quality shrimp feed in India: what do initial trials show? Bay of Bengal News September 1990. A6b

Ap A.7.a. Brown J.H. 1988. Report on the visit to Thailand to establish an overseas field station for research in marine prawn culture, May 1988. A7a

Ap A.7.b. Briggs M.R.P. 1988. Techniques and constraints of shrimp farming in Thailand. Shrimp team visit to Thailand, May 2-21 1988. A7b

Ap A.8. Smith S. 1990. Intensive shrimp farming in Thailand (1990). A8

Ap A.9. Brown J.H. 1989. A franchising scheme for shrimp farmers. Fish Farming International October 1989. A9

Appendix 2: Publications relating to section B of the project.

Ap B.1. Fox C., Brown J.H. and Briggs M.R.P. 1991. The nutrition of shrimp and prawns - a review of recent research. Recent Advances in Aquaculture IV. Blackwells Scientific, Oxford. (In press). B1

Ap B.2. Briggs M.R.P. and Brown J.H., 1991. The effects of various lipid sources, containing varied fatty acid levels, on the growth, survival and feeding efficiency of postlarval *Penaeus monodon*. Draft version, for publication within 12 months. B2

Ap B.3. Briggs M.R.P. and Brown J.H. 1991. The effect of dietary lipid and phospholipid levels on the growth, survival, feeding efficiency and carcass composition of juvenile *Penaeus monodon*. Manuscript to be submitted for publication. B3

ODA Report for Research Project R4443, NRG 522/832/8A

STUDIES ON THE CULTURE OF TROPICAL PRAWNS, (PENAEIDS) WITH PARTICULAR REFERENCE TO HUSBANDRY, DISEASE, AND NUTRITION.

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1. Objectives - [As stated in project memorandum]

The scientific objectives of the project are to perform research fundamental to specific needs of the rapidly expanding marine prawn industry in tropical developing countries. There are many serious problems appearing during this development and the research base underlying the development is very limited. Certain areas can be identified where significant improvements could be expected in culture through appropriate research. These are:-

1 A. Husbandry

In this area research will be carried out on:

Ai) the effect of water quality changes on the prawn *Penaeus monodon* under laboratory conditions. First studies will be on the effects of aluminium, followed by copper.

Aii) the factors that contribute to the frequent and widespread problem of declining prawn pond productivity, generally found after the second year of cultivation, will be investigated in conjunction with the disease component of the project. This is essentially an open ended project because it is not known whether specific factors accumulating in the sediment or disease is contributing most to the mortalities found.

Aiii) an assessment of present commercial hatchery practice will be carried out and progress will be made towards development of a dependable system that does not rely on antibiotic use as most systems do at present.

1 B. Nutrition

Investigation in this area will include:

Bi) the effects of protein; energy ratio variation and the protein sparing effect of utilising optimal lipid and carbohydrate content in the diet.

Bii) carbohydrate utilisation by prawns.

(i) and (ii) will be carried out in nutrition trials in tank systems in Stirling and will concentrate particularly on nutritional requirements of the prawns at the nursery stage.

Biii) development of practical feed formulations based on locally available raw materials {the subject of separate submissions to EEC (for Malaysia and Sri Lanka) and to ODA (with ODNRI for Bangladesh)}.

Biv) an investigation of feeding practice for post-larval rearing will be carried out. This will look at various parameters such as algal and zooplankton contributions to nutrition, artificial food and post-larval density and feeding regimes.

1 C. Disease

The project will investigate

Ci) the presence of specific and non-specific responses to pathogenic material (eg bacteria) and other antigens in the haemolymph and other tissues. (This is work funded jointly with the Wolfson Foundation)

Cii) field and laboratory work on infected material from both natural and cultured stocks will identify important disease conditions that require further study. This will be in conjunction with the environmental factors affected by husbandry which appear to have an important relationship with disease.

The work will be carried out in Stirling, at the field station in Thailand and at shrimp farms. Studies at Stirling should produce publications by the end of year 2. Overseas work will be dependant on the establishment of the field station which should be running by the end of year 1, and since the nature of the work is related to field problems it is more difficult to establish a time frame. The questions posed for the overseas work are such that significant relevant data should be obtained and be ready for publication within the three year period.

Modifications to the project

A change in emphasis was made in the main area of research under the heading of husbandry, as detailed in the report for the period December 1989 - March 1990 to investigate the effects of antibiotics in shrimp culture. This was indicated in a letter to the programme manager who agreed to its modification. This was a very timely change of emphasis since the problems relating to the use of antibiotics have become even more significant since then.

Contributing personnel throughout the period of the project

Academic staff
Dr Janet H. Brown, University funded Research Lecturer, Project Leader
Dr Kim Jauncey,
Dr Clive Fox, ODA funded Research Fellow
Mr Matt Briggs, ODA funded Research Assistant
Mr Gary Nash, University funded research fellow (until June 1989)
Dr Rhodri Jones, Wolfson Foundation funded Research fellow (until June 1989)
Dr Sandra Adams, Wolfson Foundation funded Research Fellow (July 1989 until June 1990)

Support staff

Mr Willy Hamilton, ODA funded technician
Miss Cora Johnstone, (May 1989 - August 1990)} University funded
Ms Sarah Christie MSc (August - December 1990)} " "
Ms Lorraine Wilson (from January 1991)

Research students working with Prawn Team

Ms Marlie Maclean. Part time - University funded (EC funding from March 1989)
Mr Simon Smith, NERC funded research student (Dr J.H.Brown)
Mr M. Latif. ADB Funded PhD student from Bangladesh (Dr J.H. Brown)
Mr K.N. Ganeswaran, Commonwealth Scholar (Dr K Jauncey) - graduated 1990.

Visitors to Prawn Unit during the project.

Dr Inocencio Higuera-Ciapara, Director, Division of Food Science and Technology, Centro de Investigacion en Alimentacion y Desarrollo, Hermosillo, Sonora, Mexico.
Dr Euna Moore, University of the West Indies, Barbados (twice).
Mr Marcello Buzzi, Artemia Reference Centre, Ghent.

Trainees working in the Tropical Prawn unit during the project.

Miss Marie Louise Felix, University of the West Indies, Barbados.
Dr Sufi Begum, University of Dacca, Bangladesh
Dr P. Keshnavanath, Mangalore College of Fisheries.
Dr Ali, British Council funded trainee from Bangladesh.
Mr Laxshman Wegeriya, EC funded trainee from Sri Lanka.
Dr Jean Assi, Marine Harvest International, (manager designate of shrimp farm in Cote D'Ivoire.)
Mr Abdul Aziz Al Dehaimi, FAO Fellowship, Fish Biologist at Department of Fisheries, Qatar.
Mr Muhammad Hatta Haji Mahmud, British Council. (Fisheries Officer at National Prawn Fry Production and Research Centre, West Malaysia.

2 Summary of the work carried out during the project

The initial activity of the project was directed to the establishment of a facility for marine shrimp in Stirling, a relatively straightforward aim once the university had built the shell for this expansion. The more difficult and time consuming step was to establish the overseas field station in Tinsulanonda Songkhla fisheries college, Thailand. Planning trips were involved and there were considerable problems due to not being able to have direct contact while construction was under way. The difficulties were further compounded by extremes of weather that no one could have envisaged. Notwithstanding these problems, the first trials were started in July 1989.

2 A. Husbandry

2 A. 1. The publication of the article entitled "Antibiotics; their use and abuse in aquaculture" by Dr Brown, which arose out of the concern expressed by item A(iii), generated a great deal of interest worldwide (Appendix A1). At the time this was written it was the prophylactic use of antibiotics in hatcheries that was the concern and it was not considered that it would be feasible for antibiotics to be used in grow-out in anything other than the super-intensive farms as found in Taiwan. The growth in shrimp farming and pressures on farmers from drug marketing people have led to increasing use of antibiotics and their use is implicated as one of the adverse factors leading to the collapse of the Taiwan shrimp industry (see Gary Nash's report in the report for the period April 1988- October 1988 - Appendix C1). Hence in the second year of the project, attention was directed towards working on the problem of antibiotic use to obtain preliminary information. Measurements of antibiotic residues were made using the Charm bioassay system which was initially leased by the Institute to see if it was a suitable tool for this purpose. (Appendices A2-5) Several publications have resulted from this development.

2 A.2.ii) The factors contributing to the problem of declining prawn pond productivity were not studied directly during the course of the project. This was originally included in the project as an open-ended objective because it is a very important area. It was hoped that the ponds at the Tinsulanonda Songkhla fisheries college would be available for use for studies but suitable ponds there have only been operational from mid 1990. (The first ponds constructed at the college were extreme acid sulphate ponds that have taken many years to prepare for shrimp culture). The loss of Dr Nash from the project and from his planned position on the projected ODA disease project in Thailand also put a stop to the possibility of useful collaborative research with him.

2 A.3. There was however the opportunity to gather preliminary information on water quality and productivity in shrimp ponds through the collaborative project with NRI in India. This study as part of the ODA Bay of Bengal Post Harvest Fisheries Project

was to address the problem of the lack of suitable shrimp feeds for sale in India. It was designed to identify under research conditions the relative cost-effectiveness of growing *Penaeus monodon* in monoculture using feed presented in different management schemes. An additional factor was the inclusion or exclusion of poultry manure as pond fertiliser to enhance natural pond productivity. The project involved the setting up of two separate large scale trials at CIBA, Kakdwip and at the Directorate of Fisheries for Andhra Pradesh ponds, Kakinada. This project gave very useful background data on changes in ponds under culture and on the use of fertilisers under different pond conditions but also highlighted the extreme difficulty of getting meaningful results from such pond studies. The reason why essential information on the way shrimp take food in ponds is not available is because it is very difficult to carry out controlled research in ponds when variability within a pond itself, let alone between ponds, may contribute more variation in results than the different treatments. (Appendix A 6, a & b)

2 A.4. The current situation in shrimp culture in Thailand is presented in a report prepared by NERC funded student, Mr Simon Smith who was funded to visit Malaysia on the EC prawn programme (Appendix A8). He took the opportunity to see the present activities of the shrimp farming industry in Thailand and problems can be seen to have developed markedly from the situation reported in 1989 by Mr Matt Briggs (Appendix A7b). With large areas already inoperable due to uncontrolled developments there are good reasons for concern to be felt in many such countries for the future of shrimp farming.

2 B. <u>Nutrition</u>

2 B.1. The thrust of the work in this area was towards providing information for formulating diets with optimum lipid to carbohydrate and protein to energy ratios as well as optimal protein and energy levels for post-larval/ juvenile shrimp. The effect of lipid sources and fatty acid profile was investigated first in the new recirculation system followed by a trial on lipid level and effect of phospholipid inclusion. This work is being complemented by the work of Dr M.V.Bell of the NERC Aquatic Biochemistry Unit to investigate the metabolic roles of various fatty acids in *P. monodon* by analyzing the tissue distribution of lipids in shrimp reared in Stirling in conjunction with the ODA shrimp research programme.

2 B.2. Further trials in Stirling have been on carbohydrate source and carbohydrate level but the full range of trials for this project as laid out in the report, July-November 1989 have not been possible because of time constraints.

2 B.3. The field station at Songkhla has proved a useful addition to the practical aspects of the project although not without its practical problems such as tropical storms interfering with electrical supplies. In the first series of trials, 2 trials were carried out using stocking densities up to

1000 m^{-2} and investigating the effects of habitats. Having ascertained the maximum stocking density for nursery culture without aeration, work was done on stocking density using aeration, with or without habitats, substrate, and with different commercial diets. The findings indicate that while habitats conveyed no real advantage at levels of 500 m^{-2} and below, they were advantageous above this level but substrates did not convey any advantage. Further work using cages indicated that any stunting due to culture under intensive conditions could be rapidly made good. Work was done to develop a method of stress testing for hatchery and nursery production of penaeids.

2 C. Disease

2 C.1. The disease component of the project was not directly funded by ODA but the support of ODA was a precondition for the funding of the immunological work by the Wolfson Foundation.

2 C.2. Initial work was on *Macrobrachium rosenbergii* and Dr Jones had some encouraging results demonstrating a measurable immunological response to a specific antigen. One paper has been submitted for publication from this work (Appendix C5) and two are at present in draft. Subsequent work on *P monodon* showed that unfortunately *M. rosenbergii* was not a good model for penaeids.

2 C.3. Work under Dr Adams was more directed at disease problems in shrimp, particularly due to infection with *Vibrio* spp. Initial work was to develop an enzyme linked immunosorbent assay (ELISA) to detect virulent strains of *V. alginolyticus*. This was to use during challenge experiments to monitor the progress of vibriosis in *P. monodon* and to establish the level of bacteria present in the haemolymph at various challenge doses.

2 C.4. Work was also carried out to develop an inhibition ELISA to detect the presence of molecules (lectins) which may be produced by *P monodon* following immunogenic stimulation. Particulate antigens in the form of various 'cocktails' of inactivated *Vibrio* bacteria were used in an attempt to stimulate the 'immune system' of the shrimp. The response of the shrimp was monitored at various time intervals by
a) analysing the haemolymph by indirect ELISA
b) testing the haemolymph for any anti-bacterial activity and
c) challenging control and treated shrimp with vibriosis.

3 Results of findings obtained by the project

3 A. Husbandry

3 A.1. Preliminary studies on the leaching of antibiotic from food pellets showed that loss could be as high as 50% in four hours. Results from this study were presented as a poster at the conference "Bacterial diseases of fish" held in the University of Stirling, June 1990. (Appendix A 2). The implications of this are discussed in section 4 of this report.

3 A.2. Studies carried out on the suitability of the Charm-7000 system for detecting antibiotic residues in shrimp and in fish (in conjunction with Dr Valerie Inglis) showed that minimum detection levels in shrimp for amoxycillin, tetracyclines and sulphonamides were 40, 100 and 50 mg kg^{-1} respectively (Appendix Ap A5). The investigation on the effect of oxytetracycline and sulphamethazine on weight gain and survival of *Penaeus monodon* already under stress due to infection with *Vibrio* spp in postlarval and adult shrimp showed that there was no difference in growth rate during the 4 week trial between treated and untreated groups but that survival was greater in the treated postlarval group. *Vibrio* numbers did drop more significantly in the 2 antibiotic-treated groups (adults, postlarvae) but was by no means controlled by the levels of drug administered. Levels of drug did not actually reach the level shown to be effective against vibrios by a Japanese study carried out on this problem, until the third week of the trial. The Japanese study was only 1 of 2 studies so far carried out on how antibiotics do behave in shrimp which highlights the need for work to be done in this area, (Appendix A4).

3 A.3. Water quality studies as part of the project in India showed that oxygen levels were the most crucial factor using the stocking density of $10m^{-2}$. The chemical parameters generally showed synchronous trends which implied that factors within pond treatments were having less effect on the water chemistry than any variations due to the water entering from the Hooghly river. (Appendix A6a)

3 B. <u>Nutrition</u>

3 B.1. Nutritional trials in Stirling have so far illuminated the dietary requirements for phospholipids, basal lipid source and level, carbohydrate source and given an indication of the optimum dietary lipid:carbohydrate ratios.

3 B.2. Dietary phospholipids in the form of soyabean lecithin were found to have a more significant effect on growth and survival of juvenile *Penaeus monodon* than the basal lipid source. At a 9% total lipid level, diets lacking lecithin resulted in very poor survival and feeding efficiency. Increasing dietary lecithin levels to 3% doubled shrimp survival. Any combination of 3-6% basal lipid and 3-9% lecithin (0.5-1.2% phosphatidylcholine) at a total lipid level of 6.5-9.5% resulted in optimum shrimp production. (Appendix B3)

3 B.3. Feeding different lipid sources resulted in only slightly increased growth and survival for fish oils over plant oils, probably due largely to their better fatty acid ratios, particularly in terms of high levels of long chain n-3 series fatty acids. (Appendix B2)

3 B.4. In terms of dietary lipid level, shrimp survival was poor at less than 3% total lipid, but was not adversely affected by increases up to 12% of the diet. For practical dietary formulation aimed at intensive shrimp culture, at least 3% of a

basal lipid source of both plant and fish origin should be included in diets. A total dietary lipid level of up to 12% could be used if the processing technology allows, permitting the use of high lipid levels to spare dietary protein for energy production. Lipid:carbohydrate ratios of between 4 and 10 seem optimum at 3-12% dietary lipid and 34-44% carbohydrate levels. (Appendix B3)

3 B.5. The work on carbohydrate sources has shown that chitin as the sole carbohydrate source results in very poor growth, but promotes excellent survival, suggesting a survival enhancing role for dietary ingredients such as shrimp head waste or shrimp meal. Glucose seemed as effective as a range of starches and dextrin but is less practical. Of the starches tested corn and rice starch proved optimum, indicating the useful role of these cheap ingredients at up to 36% of the diet at lipid:carbohydrate ratios of 4. The level of dietary carbohydrate optimum for shrimp growth and its role in protein sparing for energy is currently being investigated and will result in establishment of the optimum ratios of dietary energy substrates and energy levels in diets for intensive *P. monodon* culture. (Appendix B4)

3 B.6. Nutritional work in the field has so far confirmed the published dietary protein (45-50%) and lipid (7-9%) optima for juvenile *P. monodon* derived from laboratory trials, for shrimp held in intensive tank nurseries where supplemental feeding on natural productivity is limited. Additionally, high total energy levels (4.3 $Kcal.g^{-1}$), derived from high dietary protein and lipid levels were found to adversely affect shrimp production due to feed intake restriction. This suggests that lower dietary energy levels (3.7-4 $Kcal.g^{-1}$), using high (>20%) levels of carbohydrate to spare protein may present a more cost effective method of enhancing dietary quality. This is an important area for further study. (Appendix B6)

3 B.7. At the field station, results from using the intensive concrete nursery tank system showed that the maximum stocking density achievable in the absence of aeration was about 1,000 $PL_{15}m^{-2}$. This density can however, be increased to at least 2,000 m^{-2} with aeration and 20% tank volume d^{-1} water exchange. Rearing PL_{15} for 35 days at this density took the shrimp up to 0.5g mean weight at survival rates of up to 60% and FCRs of <1.5, resulting in a maximum production of 12 $g.m^{-2}d.^{-1}$. These results are consistent with published results from tank nurseries with other penaeid species and the production is considerably higher than nurseries using net cages/pens or ponds which are usually stocked at lower densities. (Appendix B6)

3 B.8. Other parameters investigated showed vertical mesh habitats to be advantageous in high density culture because of increased survival, but no advantage was conferred with the use of sand substrates. An analysis of the feeding rate revealed that shrimp would consume a pelleted diet at up to between 30 and 60% wet body weight per day, but that the water exchange rate must be increased to $40\%.d^{-1}$ to prevent water quality deterioration at this feeding rate.

3 B.9. Associated areas of shrimp culture investigated include:

1. A comparison of concrete tank and net cage nursery systems which indicated the feasibility of low-cost, low technology net cage nurseries providing similar production as concrete tanks at low stocking densities;

2. An analysis of cage ongrowing of nursed shrimp juveniles to assess the effects of nursing on subsequent ongrowing performance. Results from these trials indicated that the stunting experienced as a result of high density nursing was not carried over into ongrowing, but was compensated for within one month of ongrowing, again indicating the feasibility of nursing juvenile shrimp prior to ongrowing; (Appendix B8)

3. Stress testing of shrimp both prior to and after intensive nursery rearing. These trials established a protocol for the quick and easy determination of postlarval shrimp quality which could be easily applied to commercial conditions in order to determine whether it would be economic to stock a particular batch of juveniles. Currently, no such procedure is used and shrimp may be stocked at twice the recommended density to allow for the currently unforeseeable higher mortality of certain batches. This procedure could thus help to eliminate this unnecessary wastage. Results of the trials revealed very large quality variations between apparently identical batches of postlarvae from a single hatchery. It was also evident that shrimp produced from the intensive tank nursery were of significantly higher quality in terms of stress resistance than ex-hatchery postlarvae. Additionally, differences in postlarval quality were shown to be directly related to subsequent shrimp performance in the nursery, providing a good indication of the importance of postlarval quality testing in enhancing the efficiency of the shrimp culture industry. (Appendix B7)

3 B.11. Previous research carried out by the NERC Aquatic Biochemistry Unit has focused on the distribution of fatty acids in the phospholipids (cell membrane lipids) of various aquatic vertebrates e.g. cod and rainbow trout. This work is being extended to cover some invertebrates including shrimp with the assistance of the Tropical Shrimp unit. Neural tissues have received particular attention in these studies. As a preliminary study the eyes were removed from six prawns (*Penaeus monodon*) obtained from the stock held at the Institute of Aquaculture. These were dissected and tissues pooled into terminal ganglia, mid ganglia and optic nerves. Fatty acid profiles of total lipid extracts from these tissues were prepared. The polyunsaturated fatty acid (PUFA) content of the nervous tissue was found to be low; this is in line with findings from vertebrate tissues. The overall PUFA profiles of terminal and mid ganglia were similar. However, in contrast to vertebrate tissues so far studied, 20:5 n-3 dominated over 22:6 n-3. Previous work had indicated a possible essential role for 22:6 n-3 in vertebrate retinas with high levels of di-22:6 n-3 molecular species being present in

certain phospholipid classes. The occurrence of reduced levels of 22:6 n-3 in crustacean eyes may be related to their less developed visual function.

3 B.12. The results are sufficiently interesting to encourage further work. Samples of wild *P. monodon* are currently being obtained. It is hoped to examine the fatty acid profiles of the neural tissues in considerably more detail. The results should provide an interesting contrast to the vertebrate studies previously undertaken. In addition, it is hoped to compare the lipid profiles of *P. monodon* with those of *Pandalus borealis* from Antarctica (collected by British Antarctic Survey) and to temperate species. This forms part of a programme of NERC funded work on the relationship between temperature and membrane lipids. It is useful to be able to collaborate in this minor way since the findings may illuminate some of the problems thrown up by the more applied work of the ODA project.

3. <u>Disease</u>

3 C.1. The work on the immune system of *Penaeus monodon* was most productive. The ELISA developed to detect *Vibrio parahaemolyticus* biotype *alginolyticus*, the probable causative organism of Vibriosis in shrimp, was investigated further. The assay was found to have a sensitivity threshold of $1x10^3$ bacteria ml^{-1} and appeared to be specific for *V. alginolyticus*. Other species of *Vibrio* tested, *(V. anguillarum, V. ordalli, V parahaemolyticus*) yielded a negative response. This work has been published in Aquaculture and is in Appendix C3.

3 C.2. As indicated earlier in the report, *Vibrio* spp. are opportunist pathogens of shrimp and therefore in general, Vibriosis disease is difficult to induce in shrimp other than by direct injection. Thus challenge experiments were performed in this manner. All five strains of *V. alginolyticus* tested however, appeared to be avirulent. Fresh isolates from Vibriosis outbreaks therefore need to be sought before an effective challenge can be established. Although no mortalities occurred during challenge with strain 1318 *V. alginolyticus* was detected both by ELISA and the plate count method in eleven out of fourteen of the challenged shrimp.

3 C.3. An inhibition ELISA was developed to detect lectins which may be produced in response to exposure to killed *V. alginolyticus.*

3 C.4. Shrimp were exposed to heat-killed *V. alginolyticus* by bath or by injection. 50% of prawns injected died with 2 hours, probably due to toxic shock. The response of *P. monodon* was measured at intervals of 4 hours, 1, 2, 5, 7, and 9 days by taking haemolymph samples from three shrimp from each treatment group at each test time. Controls bathed or injected with buffer were tested simultaneously. Tests were carried out for lectins, bactericidins and haemagglutins. Clearance of bacteria from the haemolymph was also monitored.

3 C.5. Greater than 99% of bacteria were cleared from the haemolymph within four hours. A low level of bacteria persisted until day 5 in some shrimp. Bactericidins and lectins were induced one day after exposure to heat-killed bacteria. The appearance of lectins was short-lived and only detected in bacteria-treated shrimp. Bactericidins, however, peaked at day 2 and were detected until day 5. In addition, they appear to be briefly induced at day 2 in control shrimp, particularly in those which were injected. Haemagglutin activity was detected in both control and bacteria-treated shrimp at a variety of levels. This work has been published in 'Fish and shellfish immunology' and a copy is appended in Appendix C4.

4. Implications of the research for development.

4 A. Husbandry

4 A.1.The work carried out on antibiotic residues with the husbandry programme has rather serious implications for development. The dependence of many salmon farms on the use of antibiotics is already established. There is alarming evidence that shrimp farming, which still has enormous potential as a source of income for all levels of society in developing countries, could become equally dependant. The main reason for using antibiotics in shrimp farming is due to infection with *Vibrio* spp. These are generally not primary pathogens of shrimp, but merely become pathogenic when shrimp are stressed. The avoidance of high stocking densities (not more than 30. m^{-2}) and provision of adequate food together with the proper management of water quality, including what seems to be a particularly important factor, the maintenance of algal blooms within the pond, appear to provide a more effective means of avoiding disease problems than treating with antibiotics once the disease has appeared.

4 A.2. However, despite the fact that there are better ways of controlling disease, i.e. by prevention, with the use of antibiotics only as a back-up should things go wrong for some reason, many farmers are using antibiotics indiscriminately and at very high dose rates. Some farms refuse to use antibiotics but if farms surrounding them are using antibiotics it is very likely that the overall bacterial flora of the water system will change substantially and may force them into the use of drugs. This abuse of antibiotics raises very important questions in addition to those raised in the article relating to drug use in hatcheries (Appendix A1). What is the effect going to be on the trophic interactions in the ponds which will be dependant on bacteria? How do the shrimp deal with antibiotics and what are the likelihood of residues being present in marketed shrimp? The first question represents an important topic for future research but the studies on leaching of antibiotics from pellets shows that antibiotic residues in ponds are by themselves likely to be a major problem.

4 A.3. The uptake experiments showed that it is quite difficult to get sufficient levels of antibiotic into the shrimp so this raises important questions as to the effect of treating shrimp in ponds with oral antibiotic when the shrimp will in any case not be feeding well. Is the antibiotic actually being taken up by the shrimp to reach therapeutic levels?

4 A.4. The measurement of antibiotic residues in commercially available shrimp showed that they can at least take it up to detectable levels but the implications of the positive results from these surveys are not clear since many countries have not determined what limits will be set for antibiotic residues in food stuffs. The levels found were, however, more than twice those of levels set for consumption of some farmed species, i.e. lobster, catfish and salmonids by the US Food and Drug Administration, albeit in only 4 samples from 136 examined.

4 A.5. At the meeting of the OIE in Paris where these findings were presented, it was pointed out in the summing up that this paper on the antibiotic residues in farmed shrimp was the only one presented that drew attention to methods of avoiding disease rather than simply attempting to treat the disease once manifest. It is very alarming that the over dependence on antibiotics may yet make this approach as untenable in shrimp farming as it appears to be in many parts of the world with already established intensive fish farming.

4 B. Nutrition

4 B.1. Results have been obtained that will allow closer definition of shrimp diets. A dietary level of at least 3% lecithin appears to have a significant growth enhancing effect. Increasing levels higher than this may result in less cost effective diets. Information from feeding different lipid sources suggests that a mixture of plant (soyabean or peanut, but not rape seed) and fish (cod liver or capelin) oils in shrimp diets may result in good production at low cost. It appears that a total dietary lipid level of up to 12% could be used if the processing technology allows, permitting the use of high lipid levels to spare dietary protein from energy production. Lipid:carbohydrate ratios of between 4 and 10 seem optimum at 3-12% dietary lipid and 34-44% carbohydrate levels.

4 B.2. Results from the work on carbohydrate sources indicate that there may be potential in the use of shrimp head waste or shrimp meal as a dietary ingredient. There also seems considerable scope to use far higher levels of carbohydrate to provide a lower energy diet, providing considerable cost savings.

4 B.3. Results from the use of the nursery system itself indicate the economic feasibility of this type of nursery system which may offer numerous advantages to the shrimp culture industry. These include, allowing accurate estimation of shrimp numbers in the pond, (important in estimating feeding rates); limitation of mortality through the stocking of larger, higher quality, juveniles; allow the more efficient use of feed, land

and pond area by the maintenance of high stocking densities important in the intensification of shrimp culture; reduction in the grow out period allowing more harvests per year; and diversification of the industry allowing efficient small scale nurseries run by local entrepreneurs to supply high quality juvenile shrimp to the ongrowers.

4 C. Disease

4 C.1. While the best defence against disease is in the avoidance of undue stress to the shrimp, any means of bolstering the shrimps defence against infection could be a great advantage. From the work done in this project it is clear that further work is necessary to elucidate the nature of the bactericidins and lectins and to test the response to other potential vaccines. Purification and characterisation of the induced molecules would provide further information on the specificity of the reaction. The possibility that some of the induced molecules may confer protection in shrimp against vibriosis can only be tested once an effective challenge method has been established. Collection and virulence testing of *Vibrio* species from disease outbreaks is therefore of great importance to the future development of a vaccine. This was to have been the problem addressed by a PhD student from the Phillippines who unfortunately was not able to obtain funding at the last moment and who went instead to work with Dr Don Lightner in the University of Arizona. However, a student from Malaysia will now undertake this topic supervised jointly by Dr Janet Brown and Dr Sandra Adams and should be starting in September 1991.

5. Priority tasks for follow up

5 1. Many of the papers from the project have been presented at conferences and /or are already in press. A further publication has been accepted for the World Aquaculture Society Conference in Puerto Rico in June 1991. The rest of the work is still in manuscript will be submitted for publication within the next 12 months.

5 2. Conferences represent a good way to spread information especially when a variety of outlets are chosen as has been the case here, ranging from papers being presented at an International specialist disease conference, an International but generalist disease-related conference and the World Aquaculture Conference which is attended by farmers, businessmen and scientists. Practical guides are also relevant and although no manuals were planned as part of this project, a manual drawing on the experience from the nursery system will be produced as part of future work.