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FINAL REPORT ON THE PROJECT

DIAGNOSTIC AND SCREENING PROCEDURES

FOR VIRUS DISEASES OF PENAEID SHRIMP

For The Period November 1, 1984 to October 31, 1986

Grant Number NA85AA-D-SG007

Prepared By:

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Environmental Research Laboratory

University of Arizona

Tucson, Arizona 85706

Date of This Report

March 31, 1987

THE UNIVERSITY OF ARIZONA
TUCSON, ARIZONA

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SUMMARY

Emphasis in the objectives of this two-year project was placed on IHHN disease of penaeid shrimp. Other virus diseases of shrimp, while studied in this project, received significantly less effort.

IHHN disease of penaeid shrimp is caused by a non-enveloped, glycerol-tolerant, very small (20 to 22 nm diameter) icosahedral virus which is replicated in the cytoplasm of host cells. The density of IHHN, as determined by density gradient centrifugation in sucrose and cesium chloride gradients, is 1.34 to 1.40 g/ml. Electrophoresis of the proteins extracted from purified preparations of the virus showed it to have one major and two minor capsid proteins of 36, 49, and 35 kilodaltons, respectively. Analysis of the nucleic acid extracted from purified IHHN virions with ribonucleases and deoxyribonucleases gave inconclusive results. Although not completely characterized, all available data indicates that IHHN virus belongs with the Picornaviridae.

IHHN was once believed to be a disease of American penaeids, and it was suggested that it was enzootic in wild penaeids along the Pacific side of Central and South America. While most shrimp culture facilities sampled during and prior to this study in that region were positive for IHHN, samples of wild shrimp from the region were uniformly negative for the virus. However, shrimp (all Penaeus monodon) from culture facilities in Malaysia, the

Philippines, and Singapore with no history of contact with American penaeids were found to have IHHN or a related strain of the virus. This finding suggests that IHHN virus may have been introduced to American shrimp culture facilities with imported stocks of P. monodon more than a decade ago, and that it spread from one culture facility to another with transfers of contaminated postlarvae and broodstock.

Current diagnostic procedures for IHHN disease are dependent upon the histological demonstration of Cowdry type A eosinophilic proteinic intranuclear inclusion bodies in target tissues of infected shrimp that have been preserved in fixatives such as Davidson's AFA and Bouin's, or in Giemsa stained wet mounts of hemocytes from shrimp with acute infections. Asymptomatic carriers of IHHNV are diagnosed by bioassay with susceptible juvenile stages of Penaeus stylirostris, which display clinical signs and diagnostic histopathology if the virus is present. The glycerol tolerance of IHHN virus was incorporated into the bioassay diagnostic procedure for IHHN. Samples from remote geographic locations preserved and shipped in 50% glycerol (rather than frozen and shipped on dry ice) were found to provide comparable results to frozen tissues in bioassays for the virus.

A method developed for primary culture of lobster cells was adapted for use with shrimp. Primary cell cultures were successfully prepared from the developing gonads of juvenile and subadult shrimp. Although attempts at passage and subculture of shrimp primary cell cultures were not successful, the routine use

of primary cell tissue culture from shrimp was found to be feasible. Monolayers of shrimp cells exposed to IHHN virus developed cytopathic effect (CPE), with the effect being found to be time and dose dependent. These findings indicate that shrimp primary cell cultures may be applicable for diagnosis of infections and in the production of virus for future research. However, the use of primary cell cultures as a diagnostic procedure for IHHN was found to be less cost and time effective than the current methods of histopathology and shrimp bioassay.

PROGRESS TOWARDS MEETING OBJECTIVES

Objective 1:

To determine the susceptibility of various representative cultured or commercially important penaeid species to IHHN virus by: (a) sampling of cultured populations of penaeid species reared with known or suspected IHHNV carrier species, and (b) by controlled laboratory studies in which various Penaeus species that represent each of the four penaeid subgenera are exposed to IHHNV.

Progress towards meeting Objective 1:

To determine the comparative pathogenicity of IHHN virus to various penaeids, we inoculated with IHHNV juveniles of the Gulf of Mexico penaeids, Penaeus aztecus, P. duorarum, and P. setiferus, and Gulf of California penaeids P. californiensis, and P. stylirostris. While infections by IHHNV were demonstrated in all three Gulf of Mexico species, infection severity (as

determined by histology) was slight and signs of serious disease due to infection were absent in experimentally infected shrimp, even 60 days after exposure to the virus and being subjected to stressful aquarium conditions. In the two Gulf of California species, diagnosable infections were not produced in P. californiensis, while IHHNV-infected P. stylirostris held under the same conditions developed serious disease within 20 days after initial exposure to IHHNV (Table 1). A summary of these studies was published in Lightner et al. (1985, J.W.M.S.).

Also in 1984-85 a population of 1,800 juvenile P. vannamei was exposed to IHHNV and reared under "stressful" high density conditions. An identical group was handled in the same manner, but was not exposed to the virus. After 39 days, shrimp in exposed and unexposed groups were similar in average weight (0.244 and 0.246 g average weight, respectively), but different in survival (73% and 96%, respectively) (Table 2). This trend continued to the termination of the study on day-60. Histological study of samples of shrimp taken during the course of the study showed that diagnosable (although relatively mild) IHHN infections were established in the exposed population, but not in the unexposed population (Table 3). The data suggests that (under the conditions of the experiment) IHHNV can cause disease and significant mortalities in juvenile P. vannamei, but that the severity of disease in this species is mild relative to what has been observed in juvenile P. stylirostris infected with the virus.

Table 1. Summary of studies run to test the susceptibility of Gulf of Mexico and California Penaeids to IHHN virus.

Species	Initial Size	N	Route of Exposure	Study Duration	Day of 1st Positive	Severity Index (*) During Max. Incidence Observed Average	N	Range
P. californiensis	4-12g	16	Oral, injection	28 days	N/A	0	10	--
	0.1g	25	Oral	28	--	0	--	--
P. duorarum	3-6g	24	Injection	53	36	3.1	7	0-10
	3-6g	25	Oral	63	--	0	--	--
P. aztecus	0.5-2g	30	Injection	53	53	5.4	5	0-9
	0.5-2g	25	Contact	53	53	3.4	12	0-8
	0.5-2g	32	Oral	63	--	0	--	--
	2-3g	13	Oral	106	65	0.9	7	0.4
P. stylirostris	0.1g	25	Oral	28	13	9.9	25	8-14
P. setiferus	0.05g	100	Oral	60	30	1.6	28	0-8

(*) Severity index = sum of qualitative rating of infection from seven organs (with infection suspect = 1; light infection = 2; moderate infection = 3; and heavy infection = 4). Maximum rating possible is 28.

Table 2. Data summary of an experiment run at the ERL-Tucson isolation wet-lab to investigate the effects of IHHNV infection on growth and survival in juvenile P. vannamei.

	IHHNV EXPOSED				UNEXPOSED			
DAYS PI	SAMPLE SIZE	AVERAGE WEIGHT	POPUL.	PERCENT SURVIVAL	SAMPLE SIZE	AVERAGE WEIGHT	POPUL.	PERCENT SURVIVAL
0	-	56 mg	2000	100	-	56 mg	1800	100
10	100	112 mg	-	-	100	119 mg	-	-
17	100	190 mg	-	-	100	193 mg	-	-
26	100	361 mg	-	-	100	274 mg	-	-
39	1454	244 mg	1454	73	1725	246 mg	1725	96
62	542	1.26 g	542	27	633	1.42 g	633	35

Table 3. Summary of histopathological observations for IHHN lesions in juvenile P. vannamei 60 days after initial exposure to the virus.

		Frequency of IHHN Lesions				
Treatment	n	Gills	Hypodermis	Nerve Cord	Antennal Gland	Hematopoietic Organs
IHHNV-exposed	17	2 (3)*	5 (1)	1 (4)	9 (1)	3 (1)
Unexposed control	10	0	0	0	0	0

* Number in parenthesis is the number of IHHN-suspect lesions observed in addition to diagnostic lesions noted, but these lesions lacked cells with pathognomonic Cowdry type A intranuclear inclusion bodies.

Similarly, in 1985-86 we observed in three cultured populations of juvenile P. vannamei acute IHHN infections which were associated with significant losses. In every case the affected populations were being held in small tanks under stressful conditions in high density (one case reported in Lightner et al., "in press", Fish Pathology). Hence, as suggested by earlier studies (this report; Bell and Lightner, 1983; and Lightner et al., 1985, J.W.M.S.). IHHN appears to be capable of causing significant disease and mortality in early juvenile P. vannamei when infected shrimp are subjected to sufficiently stressful conditions.

Objective 2:

To determine the present distribution of IHHN disease in penaeid culture facilities in the United States and its territories by examination of appropriate samples submitted by cooperating institutions and facilities.

Progress towards meeting Objective 2:

IHHN disease is most serious in high density, intensive tank and raceway cultured P. stylirostris and P. monodon. Its effect on pond cultured populations of these species is variable, and examples have been observed of both negligible and serious epizootics due to the virus. Survivors of IHHN epizootics apparently carry the virus for life and pass it onto their progeny and other populations by vertical and horizontal transmission (Lightner, 1985 and (Lightner, "in press").

IHHN virus and the disease it causes is presently known in the Americas in hatchery-reared populations of P. stylirostris, P. vannamei, and P. monodon. Its original source and distribution in wild penaeid stocks (or other crustaceans) is not known, nor has the disease been demonstrated in wild populations. In shrimp culture facilities in the Americas, IHHN virus has been detected in Hawaii, Texas, Florida, Panama, Brazil, Belize, Honduras, and the Cayman Islands. IHHN disease has been found elsewhere in the world as well, usually in facilities which have imported American penaeids (Brock et al., 1983; Lightner, 1985, and Lightner, "in press") (Figure 1 and Table 4).

Samples of postlarvae, juveniles, and/or adults of P. setiferus, P. aztecus, P. vannamei, P. marginatus, and P. stylirostris obtained since early 1984 from commercial and experimental shrimp culture facilities in Hawaii, Florida and Texas and processed by this Laboratory for diagnosis of IHHN have been negative for the disease. During the same period, however, samples of P. vannamei from research and commercial facilities in Belize, Taiwan, and Brazil, and P. monodon from commercial facilities in the Philippines, Singapore, and Malaysia have been positive for IHHN (Table 4 and Figure 1).

Table 4. Known hosts with past geographic distribution of IHHN virus positive cases.

Host Species	Known Distribution
P. stylirostris & P. vannamei	Culture facilities in: Hawaii, Texas, Florida, Panama, Costa Rica, Ecuador, Brazil, Belize, Honduras, Cayman Islands, Israel, Taiwan, & Tahiti
P. monodon	Culture Facilities in: Hawaii, Ecuador, Tahiti, Guam, & Israel In facilities using only wild caught local broodstock: Philippines, Malaysia, and Singapore

These latter samples of IHHNV-infected P. monodon are especially important to our understanding of the natural distribution of IHHNV. Unlike previous samples of the species in which we diagnosed IHHN, these three samples came from farms that had no prior history of contact with exotic shrimp (i.e. P. vannamei and/or P. stylirostris from facilities in the Americas with a prior history of IHHN), and all three farms reportedly used only captive-wild broodstock. Furthermore, only one of the three farms commented that they had experienced survival problems with P. monodon in a pattern consistent with IHHN. Hence, contrary to our earlier hypothesis that IHHNV was probably enzootic in wild penaeids on the Pacific side of Central and South America, Southeast Asia is the only region to date in which IHHN (or a very similar disease) has been found to develop in captive-wild broodstock or in juveniles obtained from such broodstock. These data suggest that IHHN may be enzootic in wild penaeids in the IndoPacific. This finding may further suggest that IHHN virus may have been introduced to American shrimp culture facilities with imported stocks of P. monodon more than a decade ago, and that it spread from one culture facility to another with transfers of contaminated postlarvae and broodstock.

Following its discovery in 1981, IHHN disease was successfully eradicated by early 1984 from four of the five Hawaiian penaeid culture facilities then in existence. All samples obtained and processed by our Laboratory from four of the five shrimp operational shrimp culture facilities in Hawaii since the 1983-1984 eradication (consisting of a whole facility

disinfection and dry-out) effort have all been negative for IHHN. The fifth facility, while initially negative for IHHNV in the tests that followed its "dry-out" period, subsequently produced positives for IHHNV in populations of pond-reared juvenile and adult P. yannamei. State of Hawaii (using the same diagnostic procedures) personnel have monitored these same facilities for IHHN, and have also found the IHHN eradication program to have been successful in all but one facility. That latter facility completed a second eradication effort for IHHNV in early 1987.

Objective 3:

To isolate, purify, and characterize IHHNV from infected shrimp, such as P. stylirostris, or from infected crustacean cell cultures using any of the presently available or anticipated crustacean cell cultures that are found to be susceptible to IHHNV.

Progress towards meeting objective 3:

Virus Characterization:

Small quantities of IHHNV have been obtained by density gradient centrifugation in sucrose and cesium chloride gradients. Virus from these preparations is icosahedral, measures 20 to 22 nm in diameter (Figure 2), and it is 1.34 to 1.40 g/ml in density (Clerx and Lightner, 1985). In shrimp tissues, the virus occurs in the cytoplasm of infected cells and measures from 17 to 25 nm in diameter, averaging 22nm (Figure 3).

Electrophoresis of capsid polypeptides extracted from IHHNV preparations from cesium chloride gradients, showed IHHN virions to be composed of one major and two minor proteins of 36, 49, and 35 kilodaltons, respectively. Analysis of the nucleic acid of purified IHHN virions with ribonucleases and deoxyribonucleases gave inconclusive results. However, Feulgen staining of tissue sections containing IHHNV nuclear and cytoplasmic inclusions show IHHNV to not contain DNA. Hence, except for the unusually high density of IHHNV, these characteristics, along with other observations obtained by TEM and histopathology of infected tissues, are consistent with members of the picornavirus group. Hence, IHHNV is now considered to be a probable picornavirus (Lightner et al., 1983a; Lightner et al., 1985; and Clerx and Lightner, 1985). While all data obtained suggests that IHHNV is a picornavirus, its density is more like that of the parvoviruses, which are also tiny (~ 20 to 24 nm diameter single stranded DNA containing viruses), and the possibility that IHHNV may be a parvovirus cannot be completely ruled out until the nucleic acid content of a sufficiently large preparation of the virus is obtained using at least two physical/chemical techniques on the same sample.

Tissue Culture:

Considerable effort was made in attempting to routinely produce primary cell cultures from penaeid shrimp that could be used to grow IHHN virus (or other shrimp viruses) "in vitro", or that could be used to diagnose the disease if a specific cytopathic effect (CPE) occurred. In initial efforts in 1985 we

prepared primary cell cultures of penaeid hemocytes. Hemocyte cell cultures prepared by this Laboratory and exposed to IHHNV at varying times after preparation seemed to be refractory to infection by IHHNV (no CPE was noted even in cultures maintained for several weeks). Hence, after several attempts at using primary cultures of hemocytes failed to produce recognizable CPE, the technique was abandoned.

In early 1986, the techniques developed by M. Brody and E. Chang (personal communication, Univ. of CA at Bodega Bay) for the primary culture of gonadal tissue from lobsters and crayfish, was applied to penaeid shrimp. Developing gonads, the dorsal nodules of hematopoietic organ, and the heart were removed aseptically from late juvenile P. stylirostris, finely minced with surgical scissors, and incubated for 24 to 72 hr in a tissue culture media containing 200 units of collagenase/ml. The media itself was media 199, with 300 mM NaCl, 20 mM containing $MgCl_2.6H_2O$, 20 mg/L proline, 400 mg/L $NaHCO_3$, 10% fetal bovine serum, the pH adjusted to 7.5 to 7.7 with 1N NaOH, and a final osmolarity within the range 650 to 800. Following tissue disruption by this method, isolated cells were pelleted by gentle centrifugation, re-suspended in fresh tissue culture media, and grown at 28° C in plastic tissue culture containers. Monolayers of gonad cells could be routinely produced by this method following incubation for one to two weeks (Figure 4). Attempts to transfer and subculture cells from these primary cultures were unsuccessful. Cells prepared from the hematopoietic nodules and heart failed to produce confluent monolayers, and thus were not subsequently

tested with IHHNV for susceptibility to IHHN virus. Chen et al. (1986, Fish Pathology, 21: 161-166), working independently in Taiwan, followed the same approach and successfully developed a method for producing primary cell cultures from developing gonads of P. monodon.

IHHN Virus CPE in Tissue Culture:

Monolayers (> 80% confluence) of primary cultures of shrimp gonad cells were exposed to 0.22 um filtered cell-free extracts prepared from homogenates of juvenile P. stylirostris with acute IHHN (Figure 5). Development of CPE was time and dilution dependent; with the 1:100 shrimp homogenate dilution producing prominent CPE within 24 hr of exposure, while the 1:1000 dilution did not produce the same degree of CPE until after 48 to 72 hr of incubation with the shrimp cells. These tests indicate that primary cell cultures of shrimp gonads could be used to produce IHHN virus for purification and characterization work, that the development of CPE in cell cultures could be used as a diagnostic test for IHHN, and that these primary cell cultures could be used in the development of other serologic or gene probe diagnostic procedures for IHHN virus, and possibly other penaeid viruses.

Objective 4:

To improve the diagnostic methods employed for IHHN disease: (1) to simplify the procedures used in terms of the facilities, personnel, equipment, and time required, and (2) to increase the sensitivity of the diagnostic methods now employed or develop new ones (using crustacean cell cultures, ELISA, fluorescent

antibody, etc., to detect IHHNV carriers or subacutely affected individuals within apparently healthy populations).

Progress towards meeting Objective 4:

A hematological method has been developed and tested favorably for the rapid diagnosis of acute, clinical IHHN disease. In this method a hemolymph sample is drawn from obviously ill P. stylirostris, smeared on a glass slide, and stained with a modified Giemsa stain developed by this Laboratory. The presence of a particular type of cytopathic change in shrimp hemocytes has been found to provide a diagnosis of IHHN disease. Diagnoses made by this technique correlate very well to diagnoses made by traditional histologic methods (Lightner et al., 1983a & 1983b; and Lightner, 1985). Hence, a diagnosis of IHHN by this procedure appears to be valid. However, the procedure is not sensitive and it is applicable for diagnosis of IHHN only in active epizootics. However, the technique is rapid (it takes only 30 min to run versus more than two days for the histologic method), and it requires only a standard light microscope to run, making the technique practical for field use.

Improvements were made in the diagnostic procedure for IHHN in which shrimp suspected of harboring the virus are bioassayed with susceptible aged juvenile P. stylirostris. IHHNV-infected carcasses of P. stylirostris collected in November of 1981 and stored frozen at -20 °C and -70 °C have remained infectious in bioassay tests run at this Laboratory to the present time

(March 1987). However, bioassays for IHHNV on shrimp samples from remote locations have been complicated by the logistical difficulties of keeping IHHNV-suspect samples frozen during shipment to labs that do the bioassays. Often shipments have thawed before arrival, and were received in an advanced state of decomposition. Shrimp samples in this condition suspected of carrying IHHNV because of their history, gross signs, and a histopathology suggestive of IHHNV, have produced negative bioassay results. Glycerine (15 to 50%) was once recommended as a storage medium for solid tissue specimens intended for virological testing when frozen storage was impractical (Dept. Army, 1964). Another aquatic animal virus, IPN of fishes, retains its infectivity for long periods of time in tissues stored in 50% glycerol, although most other fish viruses do not (Wolf, 1970).

Hence, the preservation of IHHNV-infected shrimp tissue in 50% glycerol was tested as an alternative to frozen storage for use in shipping shrimp samples from remote locations to labs doing IHHNV bioassays (Lightner et al., "in press", J.W.A.S.). Approximately 100 IHHNV-infected clinically ill P. stylirostris juveniles of 1 g average weight were preserved in 50% glycerol (1:1 volumes of water and glycerol). After 14 days storage at ambient conditions, the glycerol-preserved carcasses were fed to 0.1 g P. stylirostris to determine if the virus remained infectious. The bioassay procedures used followed those described previously (Lightner et al., 1983; Lightner, 1985). Samples for histopathologic confirmation of IHHN disease were

taken on day 0 and after the normal incubation period of the virus (on days 15, 22 and 29 post initial exposure). Appropriate non-exposed controls were also taken and examined to verify that the "indicator" shrimp population used did not harbor the virus.

The results, summarized in Table 5, show that IHHNV retains its infectivity following storage of 14 days in 50% glycerol. Incubation period, severity of IHHN infections, and mortality rates observed in the "indicator" shrimp compared favorably with similar bioassays run at our lab using frozen carcasses.

Serologic and Gene Probe Diagnostic Methods for IHHN:

Development of rapid/sensitive diagnostic procedures that are based on serologic procedures (i.e. ELISA, fluorescent antibody, etc.) for diagnosis of subclinical IHHNV-infections have been frustrated by our inability to obtain sufficient quantities of purified IHHNV for antiserum production. Despite numerous attempts, we have found IHHNV to be unstable in the purification procedures so far tested, and this has resulted in very low yields of purified virus. Hence, no serologic or gene probe tests for IHHN were developed during the duration of this project.

Table 5. Infectivity of IHHNV to juvenile Penaeus stylirostris following storage in 50% glycerol at ambient conditions.

TREATMENT	INITIAL NUMBER	NUMBER SAMPLED	NATURAL MORTALITY	NO. SURV. TO TERM.	ADJUSTED % SURVIVAL*	HISTOLOGY RESULTS (DAYS PI) (No. IHHN + / No. exam.) 0	15	22	29
A (exposed)	25	8	17	0	12 %	0/3	3/3	2/2	2/2
B (exposed)	25	9	16	0	13 %	0/3	3/3	2/2	2/2
C (not exp.)	25	9	6	10	66 %	0/3	0/3	0/2	0/2
D (not exp.)	25	9	7	9	62 %	0/3	0/3	0/2	0/2

* Adjusted percent survival = (T + [H - (M)(H)]) / I where:
  T = the number surviving in each replicate tank to termination on day 35
  H = the number sampled for histology from each replicate.
  M = the natural mortality rate in exposed or non-exposed treatments (0.26 for non-exposed from 13 mortalities / 50; and 0.66 for the exposed from 33 mortalities / 50).
  I = the initial number of shrimp per replicate tank.

RELATED FINDINGS OF THE PROJECT

Shrimp Tumors:

Two malignant neoplasms were found in this study in shrimp that were being examined for signs of infection by IHHN or other penaeid viruses. These tumors, representing two of the three described malignant neoplasms of the Crustacea, have been described as a hematopoietic sarcoma in an adult P. vannamei (Lightner and Brock, 1987), and as an embryonic carcinoma in the grass shrimp, Palaemon orientis (Lightner and Hedrick, "in press").

Serologic Diagnostic Tests for BP and MBV:

The Hawaiian strain of BP (Baculovirus penaei) was purified from wild-caught P. marginatus and used to produce antisera in rabbits. The resulting polyclonal antiserum to BP polyhedrin and BP virus proteins was used in various serologic diagnostic tests for BP from P. marginatus from Hawaii, P. duorarum from Florida, and P. vannamei from Ecuador. P. stylirostris hepatopancreas tissue was used as the negative control. The gel diffusion and FA tests worked well with fresh or frozen tissue samples, but poorly with tissues preserved in histological fixatives.

While our ELISA procedure developed for BP diagnosis provided positive diagnostic tests for the virus in known infected tissues, it also reacted with uninfected control tissues, giving false positive results. This latter finding indicated the presence of antibody to shrimp tissue(s) in our rabbit antibody

to BP. Lewis (1987) who also investigated the ELISA procedure for the diagnosis of BP, reported a similar problem, but was able to remove the anti-shrimp antibodies with an acetone extracted homogenate of shrimp hepatopancreas.

The FA test for BP was also tested on MBV-infected P. monodon hepatopancreas tissue squashes in an attempt to diagnose MBV, and the procedure was found to provide positive diagnoses of acute infections in which polyhedra were present. Subsequent histopathology of presumed MBV infected shrimp confirmed the diagnosis provided by the FA test (Lightner et al., "in press", Fish Pathol.).

Cooperative Studies With Other Groups:

Cooperative work on various aspects of the penaeid shrimp viruses was carried out during the project with a number of domestic and foreign research groups.

With Dr. J.R. Bonami and his A. Tsing of his staff (Laboratoire de Pathologie Comparee, Univ. des Sciences et Techniques du Languedoc, Montpellier, France) we worked jointly on studies on IHHN virus, MBV (P. monodon-type baculovirus), and on a reo-like virus of P. japonicus.

With Dr. R.G. Lester and J. Paynter (Dept. of Parasitology, Univ. of Queensland, St. Lucia, Queensland, Australia) we participated in a survey for virus diseases in enhanced captive

wild penaeids and in hatchery reared stocks from various regions of the country.

With Dr. P.T. Johnson (National Marine Fisheries Service, Oxford, MD) we assisted in the preparation of a review paper on the gut-infecting forms of other decapod crustacean baculoviruses, including those three forms known from the penaeids.

With Dr. J.A. Brock (Aquaculture Development Program, Honolulu, HI) and Dr. R.M. Overstreet (Gulf Coast Research Laboratory, Ocean Springs, MS) we supplied IHHNV-free stocks of P. stylirostris for bioassays performed at their research labs.

With Dr. R.P. Hedrick (Univ. of CA, Davis, CA), Dr. S.N. Chen (National Taiwan Univ., Taipei, Taiwan), and Dr. I.C. Liao (Tungkang Marine laboratory, Taiwan) we participated in a survey of shrimp farms in Taiwan for virus diseases.

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FIGURE LEGENDS

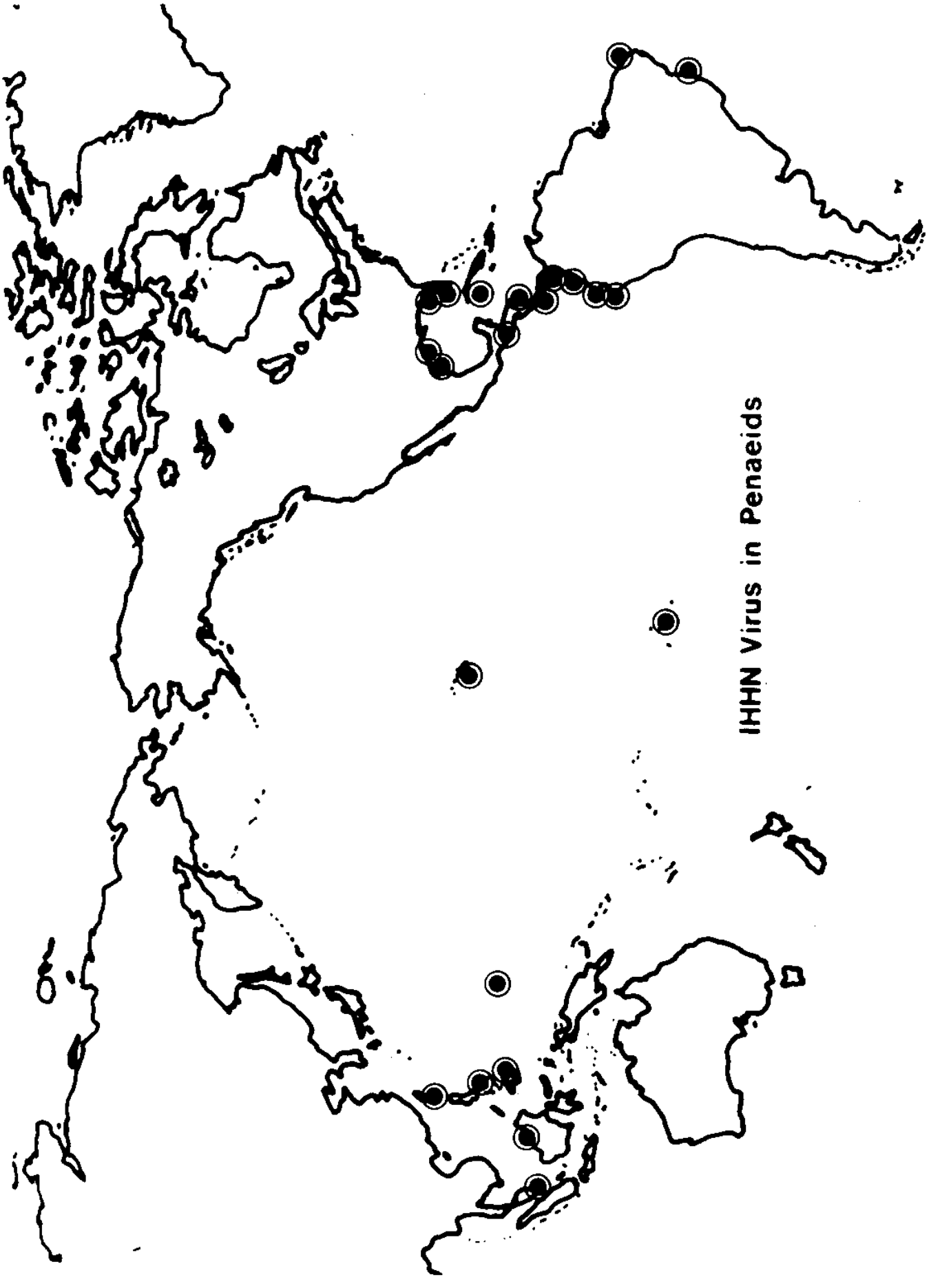
Figure 1. The distribution of IHHN virus positive shrimp in culture facilities as determined from samples obtained from mid 1980 to the end of 1986.

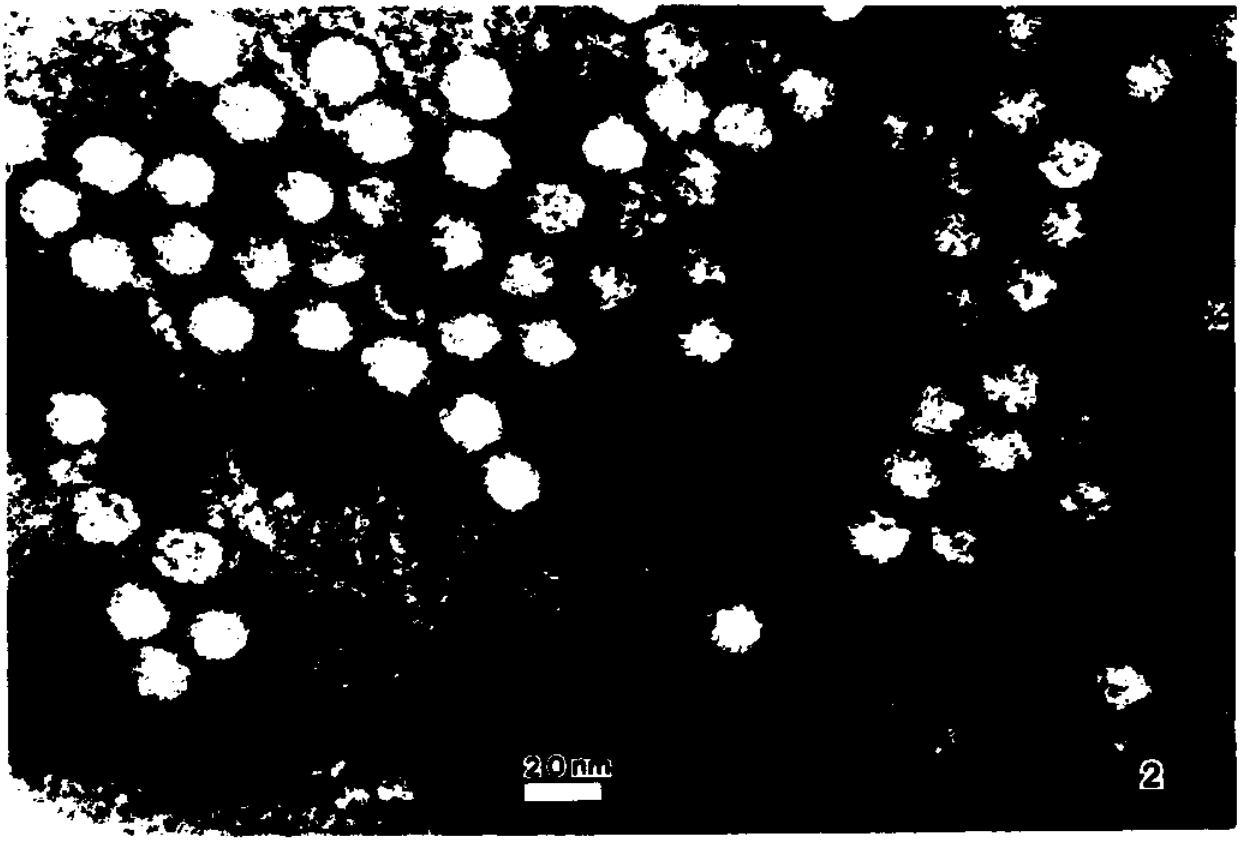
Figure 2. Transmission electron micrograph of negative stained (2% PTA) purified preparation of IHHN virions from cesium chloride density gradient centrifugation. Individual virus particles measure 20 to 22 nm in diameter. Bar is 20 nm.

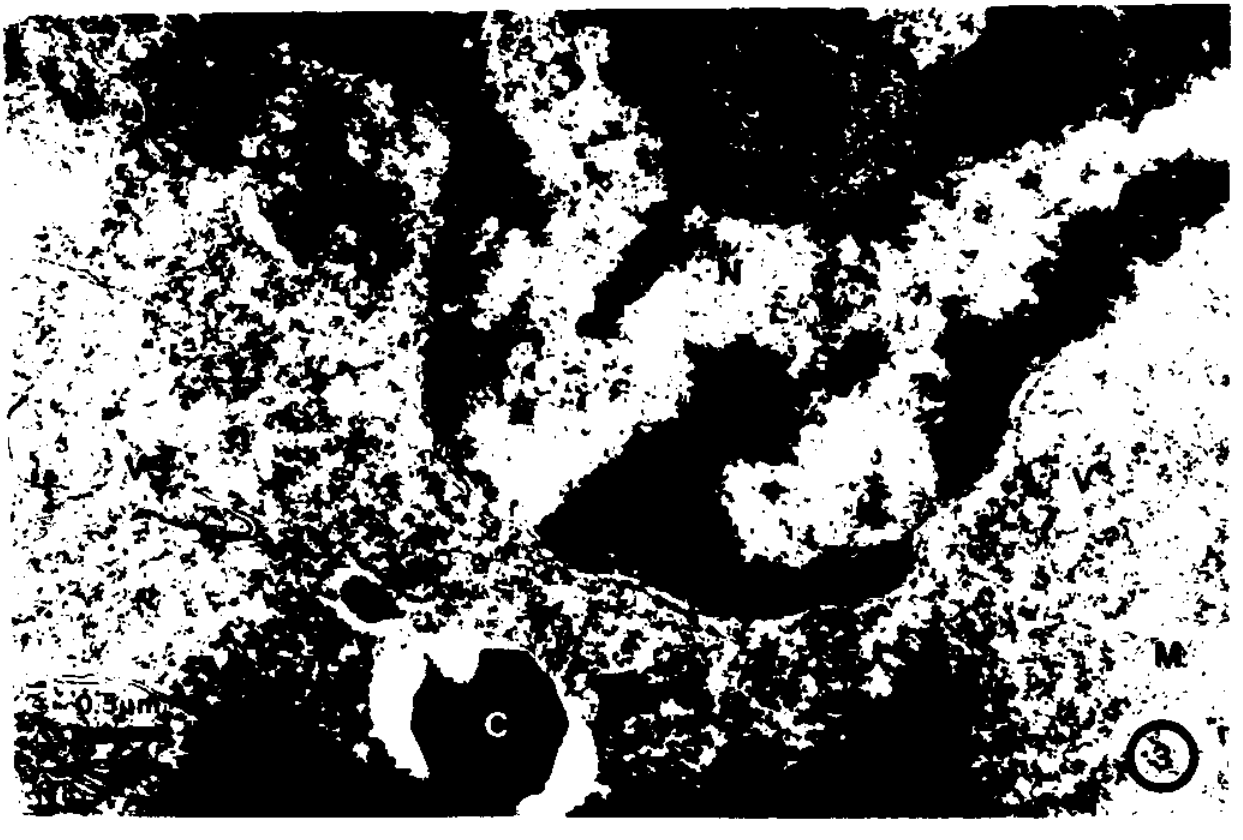
Figure 3. Transmission electron micrograph of a section of gills from and IHHN infected juvenile Penaeus stylirostris. The nucleus (N) of the gill epidermal cell shown surrounded by the cell's cytoplasm that except for mitochondria (M) is virtually filled with masses of virogenetic stroma (VS) and IHHN virus particles (V), some of which are present in paracrystalline arrays (C). Bar is 0.5 um.

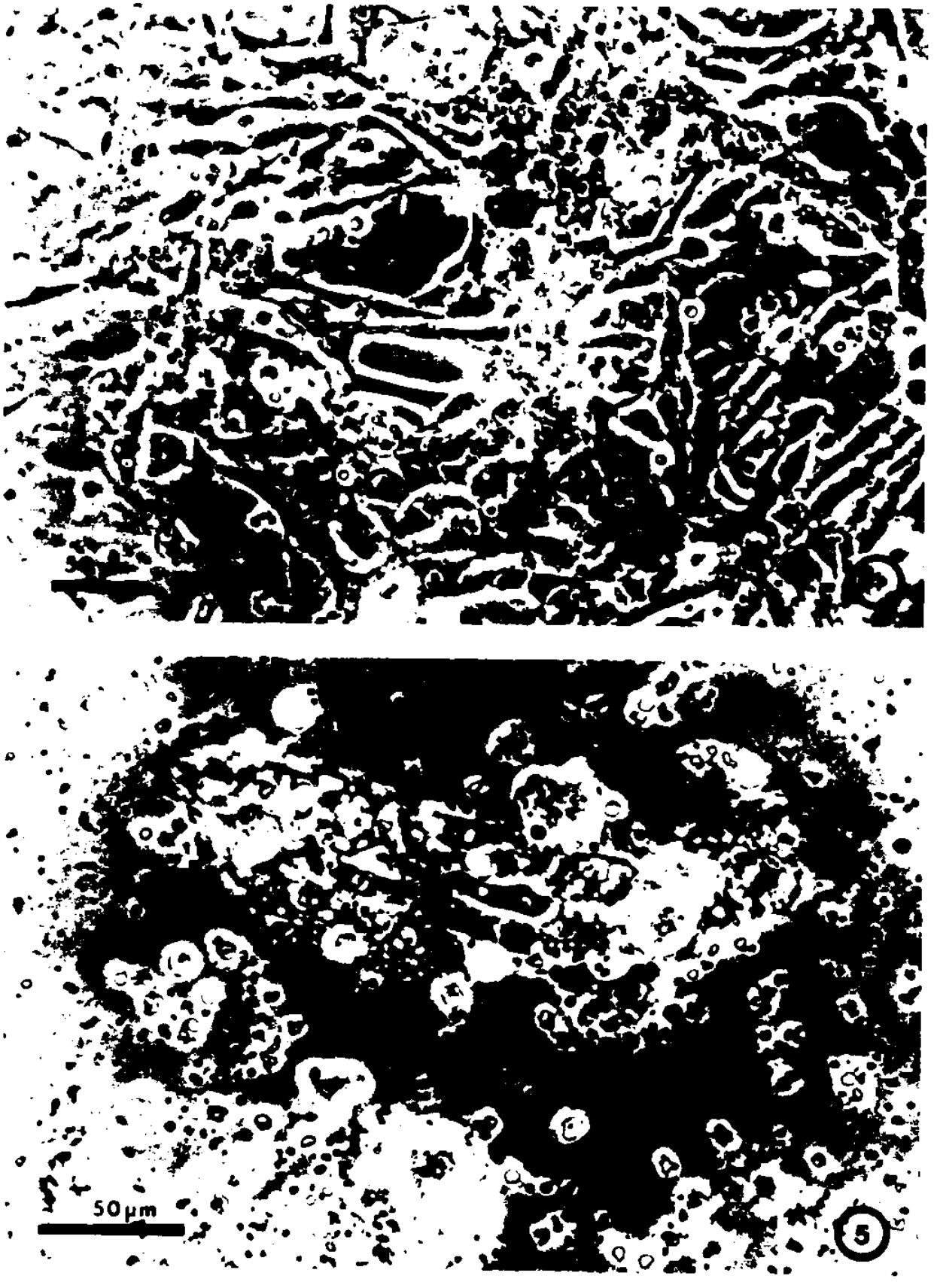
Figure 4. Phase contrast light micrograph of a primary culture of ovary cells prepared from a subadult Penaeus stylirostris. Bar is 50 um.

Figure 5. Cytopathic effect in a primary culture of shrimp ovary cells after exposure to IHHN virus. Bar is 50 um.


IHHN Virus in Penaeids


20nm
2


N
V
V
M
C
0.3μm
3


50µm
5

APPENDIX

Publications And Papers Presented That Resulted Directly Or Indirectly From This Two Year Project

Bell, T.A., and D.V. Lightner. In press. IHHN disease of Penaeus stylirostris: effects of shrimp size on disease expression. Journal of Fish Diseases.

Clerx, J.P.M., and D.V. Lightner. 1985. Physiochemical studies on three penaeid viruses: infectious hypodermic and hematopoietic necrosis virus, Baculovirus penaei and a reo-like virus from Penaeus japonicus with gut and nerve syndrome. Program and Abstracts of the Eighteen Annual Meeting of the Society for Invertebrate Pathology, p. 36. (Abstract).

Johnson, P.T., and D.V. Lightner. Submitted. The rod-shaped nuclear viruses of crustaceans: gut-infecting species. Diseases of Aquatic Organisms.

Lightner, D.V., R.M. Redman, T.A. Bell, and J.A. Brock. In press. Diagnostic methods currently in use for the penaeid viruses of potential concern to shrimp culturists in Hawaii. Proc. Marine Animal Disease and Pathology Workshop: Publ. by NMFS and State of Maine Dept. of Marine Resources. Boothbay Harbor, ME.

Lightner, D.V. 1985. A review of the diseases of cultured penaeid shrimps and prawns with emphasis on recent discoveries and developments. pp. 79-103. In: Y. Taki, J.H. Primavera, Jose A. Llobrera (editors), Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps. Published by the Aquaculture Department, Southeast Asian Fisheries Development Center, Iloilo, Philippines.

Lightner, D.V., R.M. Redman, R.R. Williams, L.L. Mohney, J.P.M. Clerx, T.A. Bell, and J.A. Brock. 1985. Recent advances in penaeid virus disease investigations. Journal of the World Mariculture Society 16, 267-274.

Lightner, D.V. 1986. Virus diseases of economically important and cultured crustaceans. p. 281. In: C.P. Vivares, J.-R. Bonami, and E. Jaspers (editors), Pathologie en Aquaculture Marine. European Aquaculture Society, Special Publ. No. 9. Bredene, Belgium. (Abstract).

Lightner, D.V., R.M. Redman, R.R. Williams, L.L. Mohney, and T.A. Bell. In press. Introductions of penaeid viruses to new geographic regions with shipments of penaeid shrimp for aquaculture. Journal of the World Aquaculture Society.

Lightner, D.V., and J.A. Brock. 1987. A lymphoma-like neoplasm arising from hematopoietic tissue in the white shrimp, Penaeus vannamei Boone (Crustacea: Decapoda) Journal of Invertebrate Pathology 49, in press.

Lightner, D.V., R.P. Hedrick, J.L. Fryer, S.N. Chen, I.C. Liao, and G.H. Kou. In press. A survey of cultured penaeid shrimp in Taiwan for viral and other important diseases. Fish Pathology.

Lightner, D.V. Submitted. Diseases of Penaeid Shrimp. In: C.J. Sindermann and D.V. Lightner (editors), Disease Diagnosis and Control in North American Marine Aquaculture. Second Edition. Elsevier Scientific Publishing Co., New York.

Lightner, D.V., L.L. Mohney, R.R. Williams, and R.M. Redman. Submitted. Glycerol tolerance of IHHN virus of penaeid shrimp. Journal of the World Aquaculture Society.

Lightner, D.V., and R.P. Hedrick. Submitted. Embryonic carcinomas in developing embryos of the grass shrimp Palaemon orientis Holthuis (Crustacea: Decapoda). Diseases of Aquatic Organisms.

Paynter, J.L., D.V. Lightner, and R.J.G. Lester. 1985. Prawn virus from juvenile Penaeus esculentus. pp. 61-64. In: P.C. Rothlisberg, B.J. Hill and D.J. Staples (editors), Second Australian National Prawn Seminar, NPS2, Cleveland, Australia.

Tsing, A., D. Lightner, J.R. Bonami, and R. Redman. 1985. Is "gut and nerve syndrome" (GNS) of viral origin in the tiger shrimp Penaeus japonicus Bate? Abstracts of the Second International Conference of the European Association of Fish Pathologists, p. 91. (Abstract).