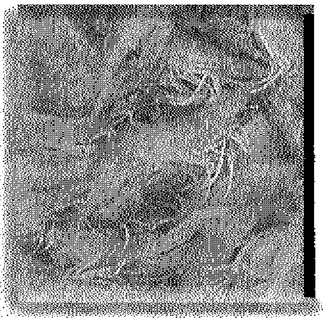

Application of Hazard Analysis Critical Control Point (HACCP) Principles as a Risk Management Tool to Control Viral Pathogens at Shrimp Aquaculture Facilities

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Publication VSG-02-10

Acknowledgement: This work is the result of research supported in part by NOAA Office of Sea Grant, U.S. Department of Commerce, under Grant No. NA56RG0141 to the Virginia Graduate Marine Science Consortium and the Virginia Sea Grant College Program

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SECTION I

INTRODUCTION

The United States is the third largest consumer of seafood products in the world, and shrimp is number two on the U.S. seafood consumption list at 1.4 kg/person (NMFS 2000). The U.S. currently imports 66% of its total edible seafood needs, approximately 1.8×10^6 metric tons (mt), valued at \$10.1 billion (US). Imported shrimp, of which approximately half are aquacultured, account for 3.4×10^5 mt, valued at \$3.8 billion (US) (NMFS 2000). In 2000, forty-eight percent of shrimp imports were shell-on heads-off shrimp (1.5×10^5 mt) valued at \$1.8 billion (US), and thirty-three percent were peeled raw shrimp (1.3×10^5 mt) valued at \$1.2 billion (US). Seventy percent of the imports came from Thailand (51%), Ecuador (6%), and Mexico (13%) (NMFS 2000).

The U.S. exports 10.0×10^6 mt of edible seafood products valued at \$3.0 billion (US), resulting in a fishery trade deficit of \$7.1 billion. Currently, 1.6×10^4 MT of shrimp are exported with a value of \$139 million (U.S.) (NMFS 2000). As the upward trend for seafood demand continues, the U.S. must develop additional sources of high valued seafood products or continue to suffer high fishery trade deficits. A successful U.S. shrimp aquaculture industry can help meet domestic demands and reduce our fishery trade deficit.

The aquaculture industry in the U.S. is maturing and under increased scrutiny and regulation. Thus, aquaculture producers must be proactive and take the time to identify all potential risks associated with their operations. An integrated risk assessment, management, and communication approach is necessary to minimize unintended consequences from aquaculture. Aquaculture enterprises should involve a holistic integrated approach addressing past, present, and future issues to ensure that aquaculture neither causes nor gives the appearance of contributing to unacceptable risks to animals and the environment. This trend for increased accountability of aquaculture with respect to animal and environmental health issues will continue (Jahncke et al., 2001).

Many disease episodes occur in conjunction with introductions of non-indigenous species. Recent episodes include the introduction of Taura syndrome virus (TSV), yellow head virus (YHV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), and white spot syndrome virus (WSSV) into shrimp aquaculture facilities around the world. TSV was identified in outbreaks in Hawaii, Texas and South Carolina. YHV and WSSV were found in a Texas shrimp farm (Lightner 1996a; 1996b). Studies in the U.S. suggest that native white shrimp may also be susceptible to these viruses (Lightner 1999). IHHNV has been found in culture facilities and wild stocks in North and South America and Asia (Brady 2000). The potential for disease transfer, specifically shrimp viruses into native wild stocks has been recognized by the Texas Parks and Wildlife Department (TPWD) as the single most serious resource management issue (McKinney 1997). Some natural resource managers have even suggested banning all live shrimp imports as a possible method to control transmission of exotic pathogens to native species (Jahncke and Schwarz, 2002).

Aquacultured shrimp

Shrimp viral pathogens can be introduced into the environment from several sources. In addition to live animal-to-animal transmission from production facilities, shrimp processing plants also represent potential transmission sources. An Environmental Protection Agency (EPA) group on shrimp indicated that there was a moderate to high potential risk for the introduction of viruses into the environment from untreated shrimp processing plant effluent and solid waste material. However, there was a low potential risk for transmission of viable viruses into the environment from effluents and solids treated at municipal sewage facilities (EPA 1998).

Shrimp processing wastewater and shrimp carapace waste material are all potential sources for transmitting exotic pathogens to wild native populations. An average shrimp processing plant can generate 3.8×10^5 liters/hour of wastewater, much of which is discharged, often untreated, into bays and estuaries. Yearly, thousands of metric tons of shrimp carapace wastes are dumped into bays or put into landfills. Leaching from landfills into water systems and transmittal of pathogens via birds feeding on carapace wastes are all potential sources of contamination (EPA 1998).

The absence of a logical risk management plan to control shrimp viral pathogens is a problem for the shrimp aquaculture industry. The Hazard Analysis Critical Control Point (HACCP) system is a risk management approach that has been accepted on a worldwide basis to prevent major food safety hazards. The Codex Alimentarius Commission has recognized the benefits of HACCP for use as a global food-control system (Garrett et al. 1997).

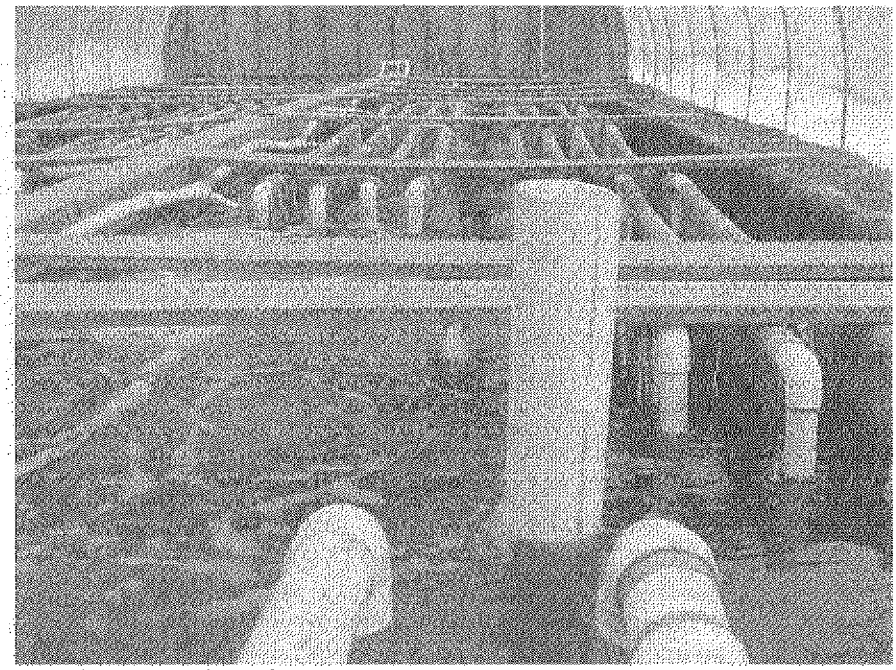
In addition to food safety, HACCP principles can be used to control a variety of non-food safety related hazards. The Food and Agricultural Organization (FAO) and the World Health Organization (WHO) also recommended that the HACCP concept be applied to fresh water aquaculture programs in Asia to control foodborne digenetic trematode infections in humans (Santos 1997). The results in Vietnam showed that application of HACCP principles to silver carp *Hypopthalmichthys molitrix* culture was effective in preventing and controlling the human intestinal parasite *Clonorchis sinensis*. Similarly, the application of these principles to fresh water aquaculture ponds in Thailand and Laos to control parasitic *Opisthorcis viverrini* infections was also successful (Santos 1997).

In the U.S., HACCP principles are also being applied as a risk management tool for a non-native oyster hatchery. (Jahncke and Schwarz 2002).

HACCP

HACCP is a preventive risk management system that requires a hazard analysis to be conducted. Critical Limits (CLs), maximum and/or minimum values, are set at each Critical Control Point (CCP) of the system. A CCP is defined as a step at which control must be applied to prevent, eliminate, or reduce a hazard to an acceptable level. When "real time" monitoring of CCPs reveals that CLs are being violated, specific corrective actions are taken to bring the system back into compliance. A specific hazard analysis is then performed on the system to evaluate the next steps. Once the cause of the violation is determined, a predetermined short-term fix is immediately implemented and a long-term solution is identified and scheduled for implementation (Garrett et al. 2000). These activities are documented through corrective action reports, including the deposition of all non-complying items.

By means of these systematic monitoring and surveillance procedures the "nice" from the "necessary" controls are separated so that major errors can be prevented. This allows focusing of resources, money, people, and equipment on essential elements of the risk management program.



Closed shrimp culture facility

HACCP TEAM

HACCP is a two-step process. The first step is to assemble a HACCP team consisting of people with specific knowledge and expertise of the operation. The team describes the product, production method, packaging, distribution, and intended users of the product. Next the team develops a flow diagram that describes the operation, followed by on-site verification of the flow diagram.

HACCP PRINCIPLES

The second step requires the team to apply the seven HACCP principles.

1. Perform Systematic Hazards Analysis
2. Determine Critical Control Points
3. Establish Critical Limits
4. Determine Appropriate Corrective Actions
5. Establish Monitoring Procedures
6. Establish Verification Procedures
7. Establish Record Keeping Systems

APPLICATION OF HACCP PRINCIPLES FOR AQUACULTURE

HACCP principles were applied as a risk management tool to control viral pathogens (listed specific pathogens-LSP) at shrimp aquaculture production sites and at a shrimp processing facility (Jahncke et al., 2001). Table 1 shows the hazard analysis for a shrimp aquaculture production facility. The operational flow diagram is shown in Figure 1. At each operational step and CCP, hazards were identified and assessed, control measures and CLs were established, and monitoring procedures and record keeping requirements were identified. Outsourced live shrimp receipt, shrimp shipment (live and finished product), feed receipt, and incoming water were identified as CCPs, as well as effluent water at the pond growout step. The preventive methods to control detectable LSPs associated with shrimp, feed, and water are:

1) To ensure receipt and production of detectable LSP free shrimp;
2) To ensure detectable LSP free water and feed ; and
3) To treat the water and feed to eliminate LSP

Preventive measures to control the detectable LSP hazards associated with employees, animals, and equipment focus on:

1) Developing and implementing employee training programs;
2) Following Good Agricultural Practices (GAPs); and
3) Developing written detailed Standard Operating Procedures (SOPs).

Appendix I provides a brief description for a closed recirculating shrimp production system. Figure 2 shows the flow diagram for a shrimp production facility. Table 3 shows the hazard analysis. Outsourced live shrimp and shrimp shipment, prepared and live feed receipt, and incoming water were identified as CCPs. Preventive measures to control detectable LSPs are:

1) Ensure receipt and production of detectable LSP free shrimp;
2) Ensure detectable LSP-free water and feed; and
3) Treat water and feed to eliminate detectable LSP.

Preventive measures to control detectable LSP associated with employees, animals, and equipment focus on:

1) Develop and implement employee training programs;
2) Follow GAPs; and
3) Develop written SOPs for the facility.

An abbreviated flow diagram for a shrimp processing facility is shown in Appendix II, Figure 3. Critical Control Points (CCPs) include shrimp receipt, protein solids recovery, transport of processing solids, treatment of processing solids, and wastewater discharge. Measures to prevent, eliminate, or reduce to acceptable levels the hazards are:

1) Review of receiving records to determine origin of shrimp;
2) Treat processing solid wastes on site, or transport wastes in a leak proof container to an off site treatment site;
3) Ensure adequate heating during dehydration of solids for detectable LSP inactivation;
4) Discharge effluents into a municipal sewage treatment system; or
5) Filter and foam fractionate effluents to reduce total solid concentrations and reduce detectable LSP concentrations.

CONCLUSIONS

HACCP principles provide a logical step-wise approach to identify and control animal and environmental hazards associated with aquaculture production and processing facilities. This management approach emphasizes process control and concentrates on points in the operation that are critical to animal and environmental safety.

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SECTION II

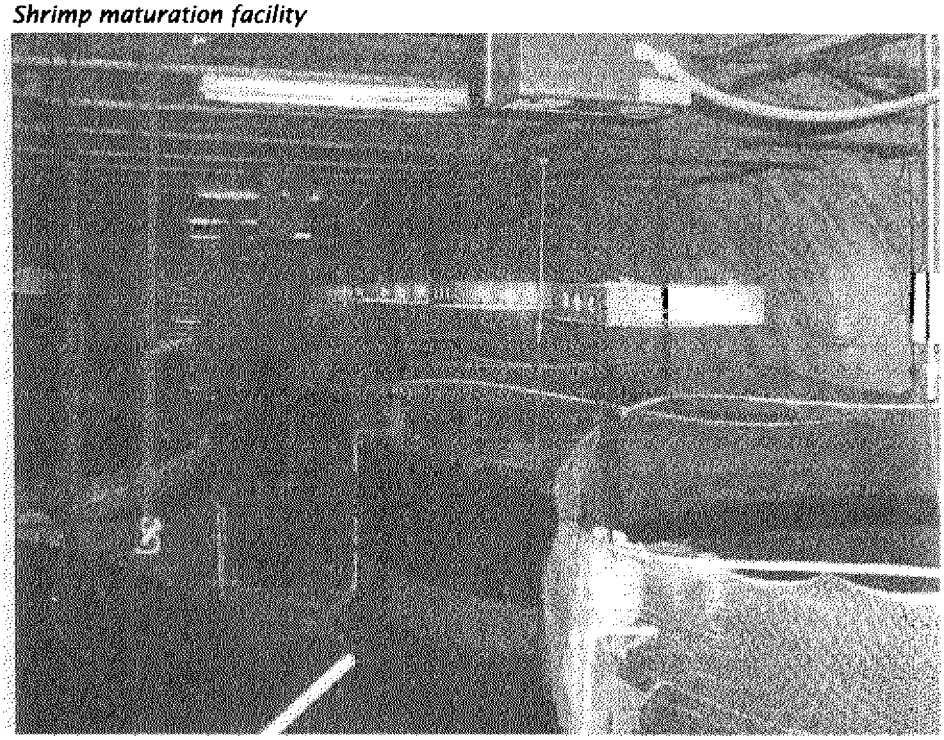
VERTICALLY INTEGRATED SHRIMP MARICULTURE PRODUCTION

Vertical integration of shrimp mariculture production has many advantages in improving control over various aspects of the production process. With the proliferation of infectious disease and increasing attention to biosecurity, control over stock quality is crucial. An increasing body of literature highlights recent advances and potential of captive breeding programs that have been developed for penaeid shrimp. Vertically integrated shrimp production enhances the potential for selective breeding and improved control over stock health, factors which will be increasingly important for risk management and overall financial success. Production phases need not be conducted at the same geographic location. There are in fact several advantages to separating various phases of the production process. Logistically, transport of broodstock and larvae is readily accomplished. Optimal water conditions vary between production phases such that a site optimal for larval production may be poor in terms of growout. Maximum biosecurity can be achieved through isolation of breeding, hatchery and production phases. The following provides a description of the major components of integrated production.

BREEDING AND BROODSTOCK PRODUCTION

The first step in production is the breeding and broodstock rearing facility. In this facility, a small-scale maturation and larval production center is coupled with a broodstock rearing component. The maturation system can be designed to allow for maximum control over breeding pairs. This requires effective tagging protocols and maturation systems sized for better control over mating rather than maximum nauplii production. Although mating between families can be controlled in a conventional maturation setup, precise control of individual parents requires unisex culture and artificial insemination. Therefore, larval culture and nursery systems should be designed to allow for a large number of batches with relatively few larvae per batch. This presents operational challenges very different from a typical commercial hatchery or nursery system. Ease of operation with large numbers of small units and uniformity between units are key components. This is especially crucial in the nursery phase where shrimp are reared from PL to a taggable size. Accurate and thorough tagging protocols, although labor intensive, are crucial for identification of progeny. Opportunities exist for quality assurance and/or cross identification using molecular technologies. The gro-

Shrimp maturation facility



Shrimp maturation facility

wout phase for broodstock is accomplished most easily at low densities in large production units with high levels of natural productivity. Ideally, production systems should be situated in isolated locations to minimize potential contamination from natural or cultured shrimp. Broodstock production in smaller indoor or enclosed units at higher densities, where biosecurity can be maintained, has been demonstrated in areas where outdoor culture would be prohibitive. At each of these stages, the facility must be designed with the strictest biosecurity protocols possible to ensure adequate controls for water, personnel and equipment. Contamination at this stage will affect all subsequent stages. Introduction of viral pathogens in broodstock can have profound effects in future generations. For example, IHHNV may be asymptomatic if introduced in the growout phase but if replicated through broodstock, serious runt deformity syndrome may result. Similarly, increased incidence of WSSV due to vertical, rather than horizontal transmission has been demonstrated. With investments in and value of stocks increasing from generation to generation, risks associated with contamination are highest at this phase of production.

MATURATION AND REPRODUCTION

Techniques for commercial scale reproduction of penaeid shrimp in captivity have been well established for over the past 15 years. The basic components of the maturation and reproduction facility include water treatment, maturation tanks and spawning tanks. Water treatment systems are designed to provide the highest quality oceanic seawater. Particle filtration, activated carbon filtration, disinfection, temperature control and addition of EDTA are typically employed. Many systems utilize a recirculation loop to reduce water usage, particularly in areas where a higher degree of filtration and/or temperature adjustment is necessary. Simplicity seems to be an advantage in these recirculation loops with avoidance of areas where organic material can accumulate as a key prerequisite. Spawning systems typically require the best water quality and some facilities add water-polishing steps for spawning tank water. While various designs have been applied, large group spawning tanks have been suggested as the most practical for mass nauplii production. An egg washing and disinfection stage and separate hatching tanks have been incorporated at many facilities to reduce the potential for transfer of pathogens between broodstock and larvae. If properly designed, a high degree of isolation can be maintained between broodstock and larvae thereby eliminating extra-oocytic vertical transfer of pathogens.

LARVAL CULTURE

Larval culture facilities typically consist of water treatment, algae production, *Artemia* hatching, larval rearing, and acclimation/packing areas. Water quality requirements and water treatment for larval culture are very similar to that applied for maturation and reproduction. Water of the highest quality is normally produced for the algal culture area where axenic cultures are maintained. Single celled algae including *Chaetocerous*, *Tetraselmis* and *Skeletonema* among others, are normally produced for the earliest larval stages. Freshly hatched *Artemia* cysts are typically fed to larvae in later stages of development. At some facilities these cysts or freshly hatched nauplii are disinfected and/or rinsed before introduction into larval culture tanks. These natural feeds are usually supplemented with one or more prepared microparticulate feeds. Many larval culture facilities now culture bacteria, which are added to the production tanks as a probiotic. Production tanks are typically designed to maintain particles in suspension as much as possible and a conical parabolic or U shaped tank may be used. High levels of natural or artificial lighting are typically encouraged. Some facilities currently employ a two-stage production strategy to maximize system efficiency and to provide optimal conditions for larvae as they mature. In the two-phase system, shrimp are transferred when they metamorphose to post larvae and assume more benthic behavior. Larger production units may be used for the second phase in which natural productivity of zooplankton and periphyton are encouraged. Larval culture facilities are typically designed to allow for periodic dry outs and cleaning and disinfection of water supply systems.

A nursery phase may be included between the hatchery and growout phases, although in most operations post larvae are stocked directly into ponds. Advantages of the inclusion of a nursery phase include the ability to head start shrimp in a more controlled and biosecure environment, allowing animals to reach a larger size before being exposed to less controlled growout pond conditions. Use of a nursery can allow for more efficient use of production space as animals can be held at higher densities during the juvenile phase, freeing larger production pond space. In areas with limited PL production or short optimal stocking seasons, nurseries can improve the outlook for larval availability and can be used to extend the growing season if designed for enclosure. Coastal nursery facilities need little water treatment from a husbandry standpoint as rich estuarine waters provide a high level of natural productivity which can boost growth, particularly that of young juveniles. This presents a challenge for the operation of biosecure nursery production units in areas where serious excludable pathogens are endemic, as treated water must be carefully enriched before stocking. In addition to natural productivity, formulated particulate diets are normally offered. These may be supplemented with live or frozen *Artemia* during early production phases. Facilities may vary from small tanks to raceways or small earthen or plastic lined ponds. One of the most important disadvantages of nurseries is mortality associated with harvest and transfer to growout ponds. This can be reduced with effective design and operational protocols.

GROWOUT

Growout facilities are typically large earthen ponds open to the external environment. As with nursery systems, natural productivity is encouraged as a supplement to formulated diets. As stocking density increases and pond size decreases (production is intensified), the importance of natural productivity decreases while the relative nutritional contribution of prepared diets increases. In general, water treatment for pond systems is limited to settling and gross particle filtration. Although there is ongoing research to develop super-intensive biosecure growout systems, consistent commercial feasibility has yet to be demonstrated. Typically, water is pumped through the growout pond during the production cycle to maintain water quality, although the trend in the industry has been towards decreased water use, particularly early in the growout cycle. Elimination of water exchange in systems with adequate supplemental aeration or very low stocking densities has been demonstrated commercially. These practices can reduce exposure to excludable pathogens or vectors associated with exchange water. Harvest and exchange effluent are normally discharged into the environment. Problems with eutrophication and cross infection between farms can be minimized through effective regional planning, site selection and farm design.

SECTION III

APPLICATION OF HACCP PRINCIPLES

FOR A VERTICALLY INTEGRATED SHRIMP MARICULTURE PRODUCTION FACILITY

HACCP principles were applied as a risk management tool to control exotic shrimp viruses at aquaculture production sites and shrimp processing facilities. Table 1 shows the hazard analysis for the shrimp aquaculture pond production site. An operational flow diagram, preventive measures, and CCPs are identified in Figure 1. For each operational step and CCP, all hazards, control measures for the hazard, monitoring, and record keeping requirements have been identified. Outsourced live shrimp receipt, shipment of shrimp, prepared and live feed receipt, and incoming water are CCPs, as well as effluent water at pond growout. The preventive methods to control LSP hazards associated with shrimp, feed, and water are:

1) To ensure receipt and production of LSP free shrimp;
2) To ensure LSP free water and feed; and
3) To treat the water and feed to eliminate LSPs.

Preventive measures to control LSP hazards associated with employees, animal vectors, and equipment focus on:

1) Developing and implementing employee training programs;
2) Following GAPs; and
3) Developing detailed written SOPs.

DEFINITIONS

HACCP: Hazard Analysis and Critical Control Point

SOP: Standard Operating Procedure

LSP: Listed Specific Pathogen

SPF: Specific Pathogen Free

Critical Control Point: A step at which control can be applied and is essential to prevent or eliminate an animal or environmental hazard, or reduce it to an acceptable level.

Critical Limit: A maximum and/or minimum value to which a biological, chemical or physical parameter must be controlled at a CCP to prevent, eliminate or reduce to an acceptable level the occurrence of an animal and/or environmental hazard.

Monitor: To conduct a planned sequence of observations or measurements to assess whether a CCP is under control and to produce an accurate record for future use in verification.

Corrective Action: Procedures to be followed when a deviation occurs.

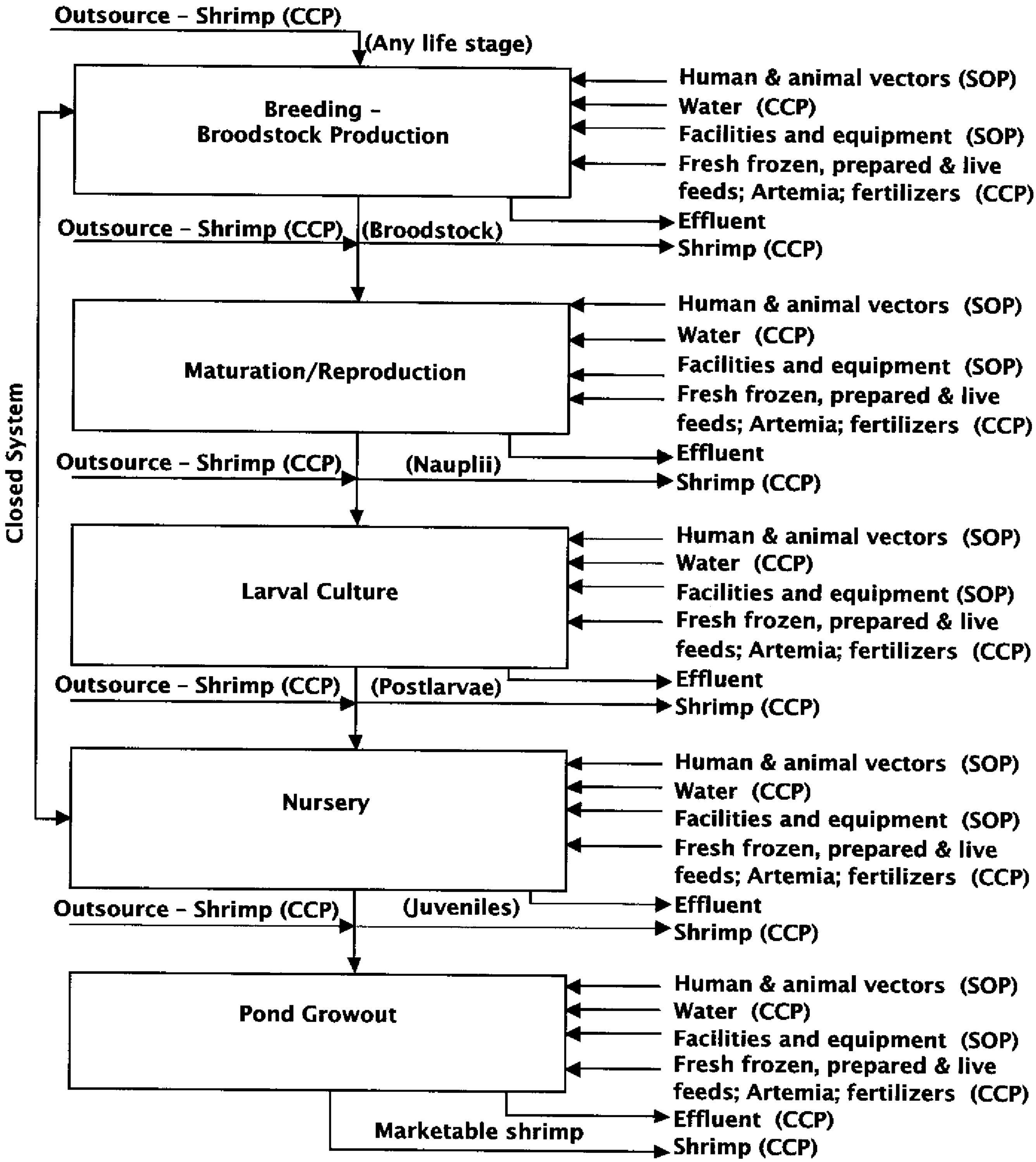
Verification: Those activities, other than monitoring, that determine the validity of the HACCP plan and that verify the system is operating according to the plan.

Table 1. Hazard analysis of a vertically integrated shrimp production facility

ID Potential Hazard	Significant	Justification	Preventive Measures	CCP
Shrimp - receipt	Yes	Shrimp may contain detectable LSP	SPF[1] certification with every receipt. Quarantineprocedures and periodic testing for detectable LSP[2]	Yes
Shrimp - shipment	Yes	Shrimp may contain detectable LSP	SPF certificate with every shipment	Yes
Incoming water	Yes	Water or waterborne particles may contain detectable LSP	Periodic testing for detectable LSP, sentinel system, disinfection of water	Yes
Fresh frozen feeds, prepared feeds, Artemia cysts, and fertilizers.	Yes	Feeds, Artemia, and fertilizers may contain detectable LSP	Certificate of compliance (COC) ensuring detectable LSP free feed fertilizers with every shipment. Periodic testing of feeds for detectable LSP	Yes
Live feeds	Yes	Water or waterborne particles with live feeds may contain detectable LSP	Certificate of compliance (COC) ensuring detectable LSP free feed fertilizers with every shipment. Periodic testing of feeds for detectable LSP	Yes
Effluent water	Yes	Effluent may contain detectable LSP	HACCP plan assures detectable LSP free status in closed systems. Water from closed systems will be discharged into municipal water treatment systems	No Yes[3]
Humans	Yes	Human vectors may transfer detectable LSP	Controlled by SOPs[4]	No
Animal vectors	Yes	Animal vectors may transfer detectable LSP	Controlled by SOPs[4]	No
Facilities and equipment	Yes	Facilities and equipment may become contaminated with detectable LSP	Controlled by SOPs[4]	No

[1] SPF - Specific Pathogen Free
[2] LSP - Listed Specific Pathogen
[3] Pond Growout effluent and any other water not discharged into municipal water treatment systems
[4] SOP - Standard Operating Procedure

Figure 1. Opperational flow diagram for a vertically integrated shrimp production facility



CCP - Critical Control Point
SOP - Standard Operating Procedure

CRITICAL LIMITS (CLs)

Significant Hazards	CLs
1. Detectable LSP on shrimp	1. No detectable LSP on shrimp
2. Detectable LSP in water	2. No detectable LSP in water or treat water with 100 ppm sodium hypochlorite for 10 minutes* or treat water with UV radiation ($9x10^5$ $\mu w_s/cm^2$) for 60 minutes* or treat water with ozone 0.5 µg/ml for 10 minutes*
3. Detectable LSP in feed	3. No detectable LSP in feed

*Chang, P-S., L-J. Chen, and Y-C. Wang. 1998. The effect of ultraviolet irradiation, heat, pH, ozone, salinity and chemical disinfectants on the infectivity of white spot syndrome baculovirus. Aquaculture 166:1-17.

SHRIMP - MONITORING

What	How	Frequency	Who
SPF certification from facility	Review of accompanying documentation	Every shipment	Facility manager
No recent history of occurrence of detectable LSP at facility	Periodic site visits and/or review of protocols and records	Yearly or as needed	Facility manager and scientists

FEED - MONITORING

What	How	Frequency	Who
Certificate of Compliance (COC) to ensure detectable LSP free feed	Review of accompanying documentation	Every shipment	Facility manager
No occurrence of detectable LSP in feed	Periodic testing of feed for detectable LSP	Quarterly	Staff scientists
Feed supplier facility/feed acquisition/ handling protocols	Periodic site visits and/or review of protocols	Yearly	Facility manager and scientists

WATER - MONITORING

What	How	Frequency	Who
Residual sodium hypochlorite concentration	Colorimetric test and/or spectrophotometer	Every batch	Facility manager or designee
UV bulb intensity	Monitor bulb life, replace as needed	Every batch	Facility manager or designee
Ozone concentration	ORP* or ozone concentration Determination	Every batch	Facility manager or designee
Contact time	Flow rate or batch holding time	Every batch	Facility manager or designee

*Oxidation Reduction Potential

CORRECTIVE ACTIONS

Where	Corrective Actions
Shrimp receipt and shrimp shipment	If an SPF certification does not accompany incoming live shrimp, reject shrimp and/or quarantine and destroy if detectable LSP positive. If outgoing shrimp test detectable LSP positive, notify customer
Feed	If a COC indicating detectable LSP free feed does not accompany frozen/fresh feeds, reject shipment
Water*	If sodium hypochlorite concentration is less than 100 ppm and contact time is less than 10 minutes**, re-treat water or if UV radiation is less than 9×10^5 $\mu w_s/cm^2$ for 60 min.**, re-treat water or if ozone is less than 0.5 µg/ml for 10 minutes**, re-treat water

*Other disinfection procedures (e.g. chloramine, micro filters) may also be appropriate

**Chang, P-S., L-J. Chen, and Y-C. Wang. 1998. The effect of ultraviolet irradiation, heat, pH, ozone, salinity and chemical disinfectants on the infectivity of white spot syndrome baculovirus. Aquaculture 166:1-17.

SHRIMP - VERIFICATION & RECORDS

Verification	Records
No detectable LSP at facility	SPF certification of facility
Periodic review of shrimp receipt and shipment records	SPF certification for each receipt and shipment
Periodic site visits	Record of site visits
Periodic review of protocols from supplier	Facility protocol records
Testing of shrimp for detectable LSP	Analytical testing records

FEED - VERIFICATION & RECORDS

Verification	Records
Detectable LSP free history of feed	Feed receiving forms
Periodic site visits	Records of site visits
Periodic review of feed shipment receipt records	COC forms for each shipment
Periodic testing of feed for detectable LSP	Analytical testing records

WATER - VERIFICATION & RECORDS

Verification	Records
Check water disinfection monitoring records	· Sodium hypochlorite concentration and contact time records · UV bulb life and contact time (water flow rate) records · Ozone concentration and contact time records
Check calibration records	· Spectrophotometric calibration records · Ozone generator calibration records
Sentinel system	Sentinel testing records

SECTION IV

SUPPORT INFORMATION FOR THE HACCP MANAGEMENT PROGRAM

1.0 General information required from the facility providing eggs, shrimp, or live feeds.

1.1 Description of biosecurity protocols at the facility.

> Provide a copy of the facility biosecurity protocols.

1.2 Description of all other aquatic species held at the facility and any pathogens associated with these species for the past two years.

> Provide a list of all aquatic species held at the facility and any confirmed pathogens associated with these species.

1.3 Analytical testing protocols and reports of analytical test results for routine surveillance disease monitoring of eggs, shrimp, and/or live feeds at the facility for the past two years.

> Provide analytical test results of routine surveillance disease monitoring of eggs, shrimp and/or live feeds at the facility for the past two years. Include information on sampling protocols and sampling frequency, sentinel test results, etc.

1.4 Analytical testing protocols and reports of analytical test results for disease outbreaks and routine disease monitoring of eggs, shrimp, and/or live foods for the past two years.

> Provide analytical laboratory reports for any documented disease occurrences at the harvest site, holding or culture facility (See Section 5.0).

1.5 Description of disinfection protocols used to address disease outbreaks within the facility within the last two years.

> Provide the written protocols for disinfection of the facility for confirmed disease outbreaks within the past two years.

1.6 Descriptions of disposition of eggs, shrimp, and/or live foods following confirmed disease occurrence during the past two years.

> Provide documentation and describe protocols used to destroy eggs, shrimp, and/or live feeds after a confirmed disease occurrence at the facility.

1.7 Analytical testing results for certification of SPF status.

> Provide analytical data to verify the SPF status of eggs, shrimp and/or live foods. Basic information that is required includes identification of the testing laboratory, a listing of the pathogens tested, a listing of the specific analytical tests performed, procedures for conducting the tests, sampling protocols and testing frequency, etc. (See Section 5.0).

2.0 Egg and Shrimp Acquisition

2.1 Source History

2.1.1 Description of the geographic source of eggs and shrimp.

> Provide a geographic description, including the longitude and latitude, of the location(s) where the eggs and/or shrimp were harvested or held.

2.1.2 Description of any disease occurrences within the past five years in the region or past two years in any facility from which the eggs and shrimp were collected or held for any period of time.

> List all documented disease occurrences for shrimp and/or eggs that occurred in the region or at the facility, where the eggs and/or shrimp were harvested, held, reared or hatched. Include documentation on any confirmed disease outbreak for any aquatic species from the region during the past 2 years.

2.1.3 Description of transport procedures for eggs and shrimp (e.g., any intermediate transfer from original source).

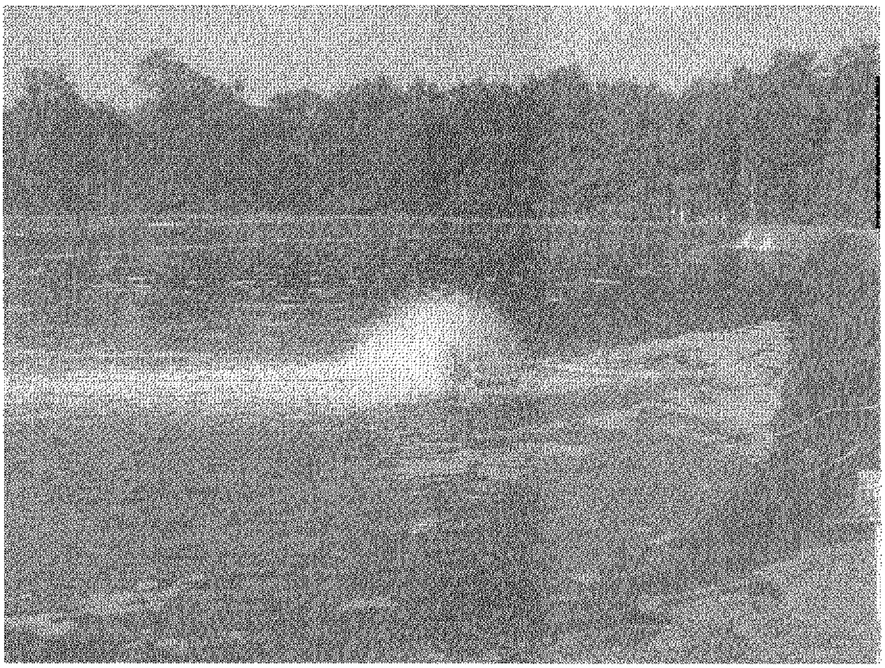
> Describe how the eggs and/or shrimp were packaged and transported. If the eggs and/or shrimp were temporarily held at an intermediate location during harvest and transport, include information on the facility, or intermediate holding site (See Section 1.0).

3.0 Live Feed Acquisition

3.1 Source History

3.1.1 Description of the geographic source of the live feeds.

> Provide a geographic description, including the longitude and latitude, of the location(s) where the live feeds were harvested or held.



Shrimp pond with paddlewheel aeration

3.1.2 Description of any disease occurrences within the past five years in the region or past two years in any facility from which the live feeds were collected or held for any period of time.

> List all documented disease occurrences for live feeds that occurred in the region or facility, where the live feeds were harvested, cultured, held, reared or hatched. Include documentation on any confirmed disease outbreak for any aquatic species from the region or facility during the past two years.

3.1.3 Descriptions of transport procedures for live feeds (e.g., any intermediate transfer from original source).

> Describe how the live feeds were packaged and transported. If the live feeds were temporarily held at an intermediate location during harvest and/or transport, include information on the facility (See Section 1.0).

4.0 Prepared Diet Acquisition

4.1 Source History

4.1.1 Description of the geographic area for diet ingredients.

> Provide a geographic description, including the longitude and latitude, of the location(s) where the industrial fish were harvested.

4.1.2 Description of diet preparation procedures (e.g., time/temperature, extrusion temperatures, etc.).

> Provide written documentation on processing procedures and temperatures used to prepare the diets.

4.1.3 Description of employee and facility sanitation protocols.

> Provide written standard operating procedures (SOPs) for employee and facility sanitation procedures at the feed manufacturing facility.

5.0 General Analytical Laboratory Requirements

> General questions that need to be addressed before selecting an analytical laboratory are the following: **1)** *Is the laboratory qualified to conduct the analyses?* **2)** *Are the laboratory personnel proficient in the analytical tests?* **3)** *Does the laboratory have the appropriate facilities, equipment and methodology to properly conduct the analyses?* **4)** *Are the proposed analytical methods accepted by the scientific community?* and **5)** *Is the laboratory accredited?*

5.1 Laboratory Protocols

General questions that need to be addressed before selecting an analytical laboratory are the following: 1) *Does the laboratory have written protocols?* 2) *Does the laboratory have an internal quality assurance program to ensure adherence to written protocols?* and 3) *Are there adequate procedures in place for sample receipt, handling and retention of the samples?*

5.2 Analytical testing results and description of specific tests

Before selecting an analytical laboratory, the following question must be addressed: 1) *Are comprehensive reports provided that document sample identification, data, methods, and interpretation of results?*

5.2.1 Specific Analytical Tests

The testing laboratory should provide a list of the specific pathogens, that they have the capability and expertise to analyze. Written SOPs should also be provided that describe the analytical test procedures used for each specific pathogen.

6.0 Shrimp Production Facility

6.1 Production Facility Protocols

See Section 1.0.

6.2 Description of visitor policy procedures

Written protocols need to be developed concerning visitors to the shrimp production facility. These protocols should at a minimum include identifications of building and other locations that are off limits to all visitors. Restrictions must be in place for any visitors that work at, or have recently visited hatcheries or other aquatic facilities.

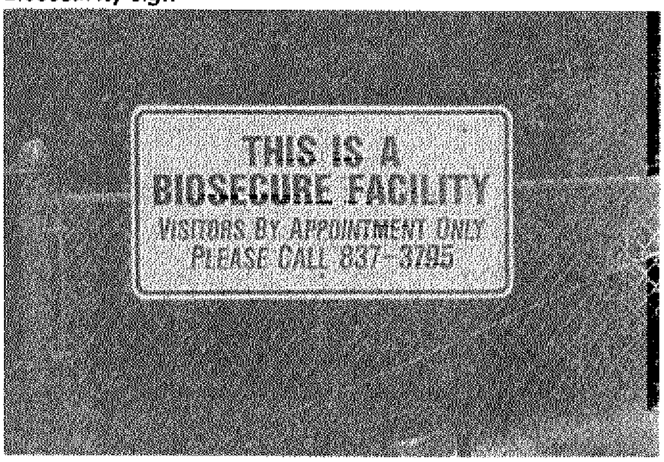
6.3 Shrimp and Egg Receipt Procedures

See Sections 1.0, 2.0, 3.0, 4.0 and 5.0. In addition, a Specific Pathogen Free (SPF) Certification must be included with each shipment.

6.4 Live Feed Receipt Procedures

See Sections 1.0, 2.0, 3.0, 4.0 and 5.0. In addition, a Specific Pathogen Free (SPF) Certification must be included with each shipment.

Biosecurity sign



6.5 Prepared Diet Receipt Procedures

See Sections 1.0, 2.0, 3.0, 4.0 and 5.0. In addition, a Specific Pathogen Free (SPF) Certification must be included with each shipment.

6.6 Disease Monitoring and Testing Protocols.

Written SOPs are needed to identify the specific diseases, and a description of sampling and testing protocols for these diseases in eggs, shrimp, live foods, water, etc. The SOPs should also include information on sentinel test locations. If analytical testing for specific pathogens is conducted at the facility, descriptions of sampling protocols, sampling frequency, analytical testing procedures, and analytical test results are also needed.

6.7 Incoming Water Treatment Protocols

Describe the type of water source, and develop written standard operating procedures (SOPs) on water disinfection/ treatment of incoming water at the facility. The treatment protocols must be validated to determine their effectiveness, and results of the validation studies must be kept on file. SOPs also need to include information on routine monitoring procedures to ensure the effectiveness of the disinfection/treatment protocols. Records on monitoring results must be kept on file.

6.8 Effluent Water Treatment Protocols

Describe the effluent water composition and location, and develop written protocols and SOPs on water effluent disinfection/treatment procedures at the production facility. The treatment protocols must be validated to determine their effectiveness, and results of the validation studies must be kept on file. SOPs also need to include information on routine monitoring procedures to ensure the effectiveness of disinfection/treatment protocols. Records on monitoring results must be kept on file.

6.9 Pond Disinfection Protocols

Describe the pond structure and layout, and develop written SOPs on pond dry out procedures. Treatment protocols must be validated to determine their effectiveness, and results of the validation studies must be kept on file. SOPs also need to include information on routine monitoring procedures to ensure the effectiveness of disinfection/ treatment protocols, and records on monitoring results must be kept on file.

6.10 Employee Policies

All employees should receive training on biosecurity protocols. Access to the shrimp facility should be limited to essential personnel only. Written protocols and employee training programs are needed to restrict movement of employees within and between buildings. Specific SOPs are also needed for equipment use and disinfection, use of employee showers, use of footwear covers, required clothing changes, etc. before admittance into biosecure areas.

6.11 Equipment Policies

Develop written procedures for the use, storage, and disinfection of equipment, tanks, nets, buckets, forklifts, carts, etc. In some instances, these objects can be color coded to control cross contamination between facility operations. SOPs also need to be developed that include information on routine monitoring procedures to ensure the effectiveness of disinfection/treatment protocols. Records on monitoring results must be kept on file.

6.12 Building Access

Color-coded signs may be placed on buildings to control access by unauthorized personnel into biosecure areas. Written protocols should be developed to restrict and control employee traffic in production buildings.

6.13 Building Disinfection Protocols

Written SOPs are needed describing how to disinfect buildings after a confirmed disease outbreak, and how to verify that the disinfection procedures were effective.

6.14 Facility Maintenance Protocols

Written SOPs are needed to ensure that all buildings, equipment, etc. are maintained on a regular basis to ensure that they are in good condition. A pest control program should also be in place to control all facets of pest control within the production buildings and on the outside grounds.

APPENDIX I

HACCP PLAN FOR A COMPLETELY CLOSED RECIRCULATING MARINE SHRIMP AQUACULTURE FACILITY

See the HACCP Plan developed for the Vertically Integrated Shrimp Mariculture Production Facility.

Except for the pond grow out step, the HACCP plan for the RAS facility is identical to the vertically integrated marine shrimp mariculture production facility. Water from grow out ponds are normally discharged into the environment and thus the growout pond water effluent is a CCP. In this example, water effluent from the RAS facility is discharged directly into a municipal sewage treatment system. However, if water effluent is not discharged into a municipal treatment system, the water effluent is a CCP.

SHRIMP PRODUCTION IN CLOSED RECIRCULATING AQUACULTURE SYSTEMS

Recent advances in biological and nutritional requirements of *Penaeid* species in conjunction with improvements in recirculating aquaculture systems (RAS) has improved the potential for culturing marine shrimp species in completely closed production systems. However, the high stocking densities and eutrophic culture conditions associated with RAS provide an optimum environment for pathogen growth and survival. Thus, due diligence must be practiced to ensure optimum water quality in order to minimize stress and disease outbreaks in cultured shrimp.

Recirculating aquaculture systems (RAS) can be defined as a collection of parts and devices used in the growing of aquatic organisms in which water is continually cleaned and recycled. They are a collection of devices used in the husbandry of aquatic organisms, and are analogous to swine barns or broiler houses. They allow multiple reuse of water within a tank system, allowing high-density production in a limited space.

There are several inputs that are critical for the successful culture of shrimp in RAS.

Water: The water is critical as diseases, toxins and other contaminates may be transferred by the water. It is recommended that RAS facilities are sited where clean, potable and sufficient quantities of groundwater is available. In addition, any water effluent dischared from the production facility should go through a municipal water treatment system, or an equivalent type system, before being released into the environment.

Feed: Feed is also a critical component in the production of shrimp in RAS. Feed must be certified as specific pathogen free (SPF). It must also be comprised of high quality ingredients and be nutritionally complete for all stages of shrimp production.

Outsourced Shrimp: Shrimp brought into the production facility from an outside supplier must have accompanying documentation and testing results to ensure receipt of SPF free shrimp. In addition, quarantine and analytical testing protocols must be in place at the receiving facility to ensure acceptance of only SPF shrimp.

GROWOUT PRODUCTION SYSTEM

The shrimp production system described in this section is designed to minimize construction costs and maximize production capabilities. The prototype tank has 3.8 cubic meter capacity with an additional 0.4 cubic meters for the water treatment equipment. Double drain technology is used with a water flow of 98 l/min via airlift manifolds from the culture tank directly to air driven Rotating Bio Contactors (RBCs). The used water effluent travels from the double drains (water flow rate of 98 l/min) into a swirl separator (10 min. retention), followed by a custom designed protein skimmer treatment (gas flow rate of 10 l/min). [**Note**: the skimmers can be operated with injected ozone. Approximately 25 g of ozone is used per kg of feed. The amount of ozone in the system is monitored using oxidation/reduction potential (O/R) controllers.] The water returns to the RBC and then back into the culture tank via an air lift manifold. The water is turned over every 36 min. The RBC has a 929 sq. cm surface area and is self cleaning.

PRODUCTION SYSTEM OPERATION

The water quality in RAS should be monitored daily. Water quality parameters that should be monitored daily include: ammonia, nitrite, nitrate, pH, alkalinity, temperature, dissolved oxygen, etc. If ozone is used in the system, additional testing for ORP, total chlorine should also be conducted. The swirl separator is drained and refilled once per day, and water movement is via airlifts.

HARVEST

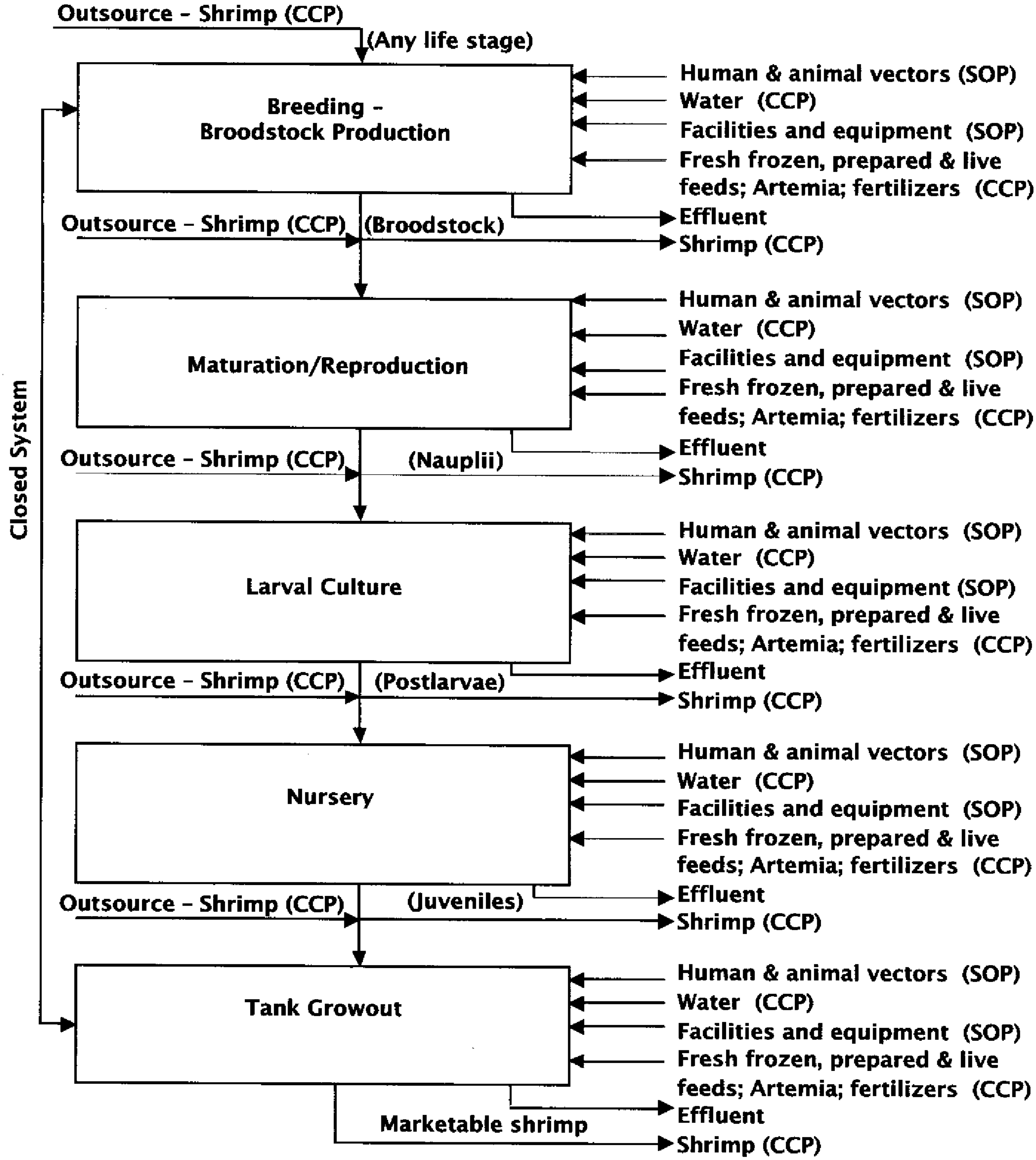
The day prior to harvest, feed is withheld to allow the shrimp to evacuate their digestive tracts. The harvested shrimp are immediately chill killed, and then sorted by size in a refrigerated packing house. The shrimp are iced, boxed and then shipped.

Table 2. Hazard analysis shrimp RAS production facility

ID Potential Hazard	Significant	Justification	Preventive Measures	CCP
Shrimp - receipt	Yes	Shrimp may contain detectable LSP	SPF[1] certification with every receipt. Quarantine procedures and periodic testing for detectable LSP[2]	Yes
Shrimp - shipment	Yes	Shrimp may contain detectable LSP	SPF certificate with every shipment	Yes
Incoming water	Yes	Water or water-borne particles may contain detectable LSP	Periodic testing for detectable LSP viruses, sentinel system, disinfection of water	Yes
Fresh frozen feeds, prepared feeds, Artemia cysts, and fertilizers	Yes	Feed, Artemia, and fertilizers may contain detectable LSP	Certificate of Compliance (COC) ensuring detectable LSP free feed with every shipment. Periodic testing of feeds for detectable LSP	Yes
Live feeds	Yes	Water or water-borne particles with live feeds may contain detectable LSP	Certificate of Compliance (COC) ensuring detectable LSP free feed with every shipment. Periodic testing of feeds for detectable LSP	Yes
Effluent water	Yes	Effluent may contain detectable LSP	HACCP plan assures detectable LSP free status in closed systems. Water will be discharged into municipal water treatment systems.	No Yes[3]
Humans	Yes	Human vectors may transfer detectable LSP	Controlled by SOPs4	No
Animal vectors	Yes	Animal vectors may transfer detectable LSP	Controlled by SOPs	No
Facilities and equipment	Yes	Facilities and equipment may become contaminated with detectable LSP	Controlled by SOPs	No

[1]**SPF** - Specific Pathogen Free
[2]**LSP** - Listed Specific Pathogen
[3] -Only if water is not discharged into a municipal water treatment system
[4]**SOP** - Standard Operating Procedure

Figure 2. Operational flow diagram closed recirculating shrimp production facility.



CCP - Critical Control Point
SOP - Standard Operating Procedure

APPENDIX II

Shrimp processing wastewater and shrimp carapace waste material are all potential sources for transmitting LSP to wild native shrimp populations. An average shrimp processing plant generates approximately 3.8 x 10^5 liters/hour of wastewater, much of which is discharged, often untreated, into bays and estuaries. Yearly, thousands of metric tons of shrimp carapace wastes are dumped into bays or put into landfills. Leaching from landfills into water systems and transmittal of pathogens via birds feeding on carapace wastes are all potential sources of contamination (EPA 1998).

An EPA shrimp processing group indicated that there was a moderate to high potential risk for the introduction of detectable LSP into the environment from untreated shrimp processing plant effluent and solid waste material. However, there was a low potential risk for transmission of detectable LSP from processing plant effluents and solid waste material treated at municipal sewage facilities (EPA 1998).

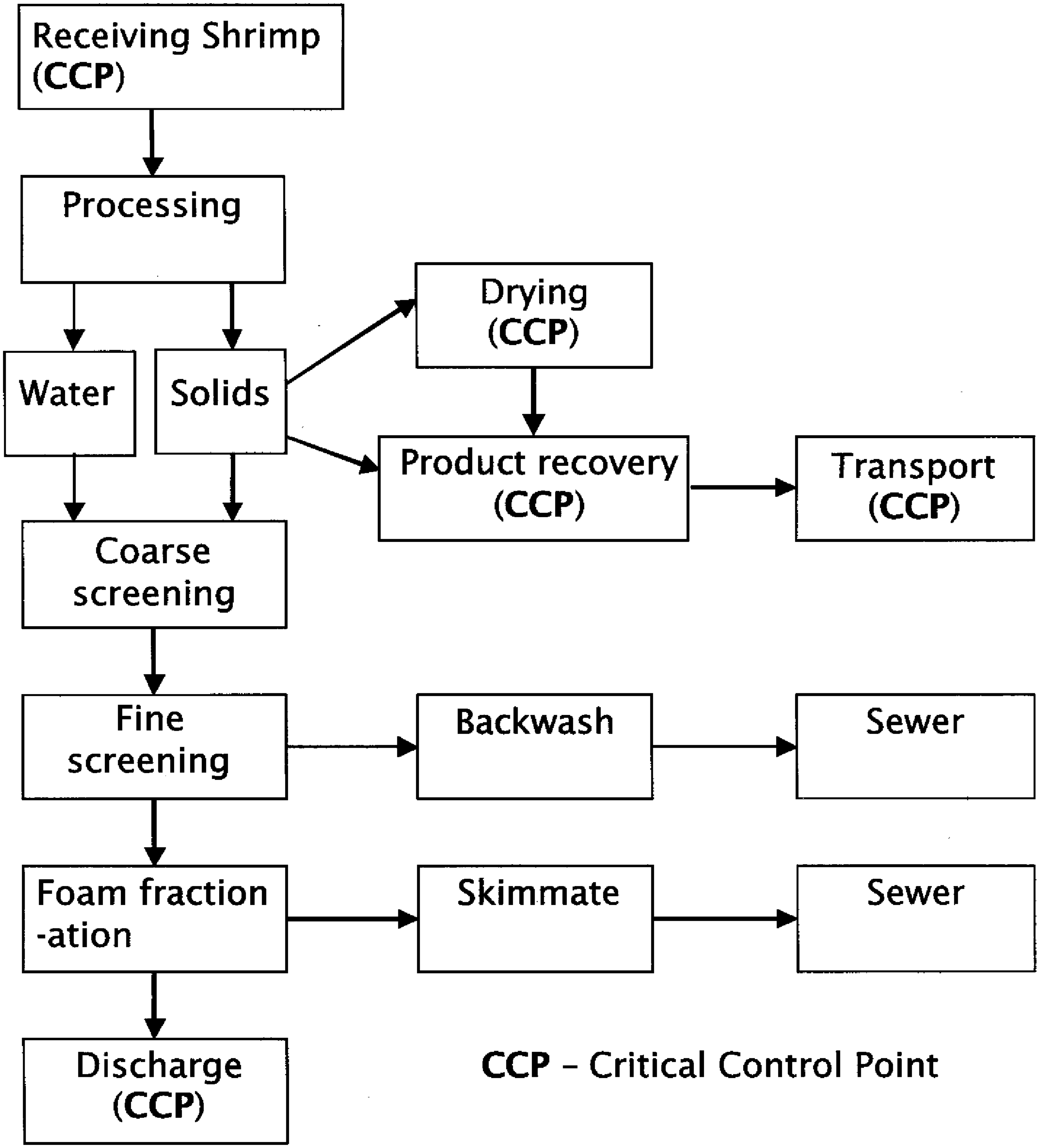
An abbreviated flow diagram for a shrimp processing facility is shown in Appendix II, Figure 3. Critical Control Points include shrimp receipt, protein solids recovery, transport of processing solids, treatment of processing solids, and wastewater discharge. Measures to prevent, eliminate, or reduce to acceptable levels the hazards are:

1) Review of receiving records to determine origin of shrimp;
2) Treat processing solid wastes on site, or transport wastes in a leak proof container to an off site treatment site;
3) Ensure adequate heating during dehydration of solids for detectable LSP inactivation;
4) Discharge effluents into a municipal sewage treatment system; or
5) Filter and foam fractionate effluents to reduce total solid concentrations and reduce detectable LSP concentra tions.

REFERENCE

Environmental Protection Agency (EPA). 1998. Report on the shrimp virus peer review and risk assessment workshop. Developing a qualitative ecological risk assessment. Eastern Research Group Inc. 110 Hartwell Avenue, Lexington, MA 02173. Work assignment No. 1-06, Under Contract No. 68-C6-0041. June 30, 1998.

Figure 3. Example of a condensed operational flow diagram for a shrimp processing company.



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