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REPORT ON THE SHRIMP VIRUS PEER REVIEW AND RISK ASSESSMENT WORKSHOP

Developing a Qualitative Ecological Risk Assessment

National Center for Environmental Assessment
Office of Research and Development
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Washington, DC

DISCLAIMER

DEDICATION

Ned Alcathe's valuable experience contributed to the development of the Joint Subcommittee on Aquaculture (JSA) Shrimp Virus Report. Early in the JSA Shrimp Virus Work Group's deliberations, Ned assisted in identifying issues and concerns regarding the potential role of shrimp processing in the shrimp virus problem. Despite being seriously ill, Ned joined the group of experts who participated in the January 1998 Shrimp Virus Peer Review and Risk Assessment Workshop; only a few weeks later, he died unexpectedly. In tribute to his technical contribution, his commitment to resolving this complex issue, and his warm, gentle spirit, we dedicate this document to Ned.

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FOREWORD

Environmental protection in the 1990s is pervaded by the language of risk, and environmental policies are set by the concepts and methods of risk assessment. Risk assessment and risk management provide the primary framework for decision-making at the U.S. Environmental Protection Agency (EPA), and EPA's primary mission is to reduce risks to environmental stressors. EPA's first Agencywide guidelines for ecological risk assessment, published in May 1998, provided a broad framework applicable to a wide range of environmental problems associated with chemical, physical, and biological stressors. However, although EPA has considerable experience in applying the ecological risk assessment paradigm to chemical contaminants, Agency experience for physical and especially biological stressors is limited. This report illustrates the applicability of the new ecological risk assessment guidelines to biological stressors such as nonindigenous pathogenic shrimp viruses.

Conducting the shrimp virus assessment illustrates several important points about the ecological risk assessment process. First is the importance of stakeholder involvement. Given that the shrimp virus issue involves sensitive socioeconomic and political issues, it was essential to hold meetings with stakeholders prior to completing the risk assessment and to conduct the risk assessment process openly. Second, although there are critical data gaps and uncertainties surrounding the shrimp virus issue, the ecological risk assessment process facilitates clear communication of available scientific information in a way that facilitates environmental decision-making. Finally, a primary objective of conducting a risk assessment is to support risk management activities. The use of this shrimp virus risk assessment as input to a subsequent risk management workshop provides this critical linkage. Overall, this assessment provides an excellent prototype for evaluating the risks associated with biological stressors.

William H. Farland
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PREFACE

Public concerns over the potential introduction and spread of nonindigenous pathogenic shrimp viruses to the wild shrimp fishery and shrimp aquaculture industry in U.S. coastal waters have been increasing. Although these viruses pose no threat to human health, outbreaks on U.S. shrimp farms, the appearance of diseased shrimp in U.S. commerce, and new information on the susceptibility of shrimp and other crustaceans to these viruses prompted calls for action. In response, the Joint Subcommittee on Aquaculture (JSA) tasked a Federal interagency Shrimp Virus Workgroup with assessing the shrimp virus problem. Four Federal agencies are represented on the JSA Shrimp Virus Workgroup: the National Marine Fisheries Service (NMFS), the Animal and Plant Health Inspection Service (APHIS), the Environmental Protection Agency (EPA), and the Fish and Wildlife Service (FWS).

In June 1997, the Shrimp Virus Workgroup summarized the available information on shrimp viruses in a report to the JSA entitled "An Evaluation of Potential Shrimp Virus Impacts on Cultured Shrimp and on Wild Shrimp Populations in the Gulf of Mexico and Southeastern U.S. Atlantic Coastal Waters" (JSA Shrimp Virus Report [JSVR]). During July 1997, in cooperation with the JSA, EPA's National Center for Environmental Assessment (NCEA) sponsored a series of four stakeholder meetings to gather stakeholder input on the JSVR and the shrimp virus issue. The JSVR and the stakeholder (public) comments formed the basis for the shrimp virus peer review and risk assessment workshop, held during January 1998. Workshop participants considered several potential pathways of nonindigenous pathogenic shrimp viruses to wild shrimp populations, including shrimp aquaculture, shrimp processing, and "other" sources and pathways, and independently assessed risks using a qualitative risk assessment approach developed by the Aquatic Nuisance Species Task Force. The workshop report was revised based on comments provided by an external scientific review in July 1998.

This workshop report, together with the results of the independent scientific review, was used as the basis for a risk management workshop on shrimp viruses held on July 28-29, 1998, in New Orleans. The risk management workshop, jointly sponsored by the EPA Gulf of Mexico Program, NMFS, and the USDA Agricultural Research Service, developed options and strategies for managing the threat of shrimp viruses to cultured and wild stocks of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters.

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1. EXECUTIVE SUMMARY

This report highlights issues and conclusions from the Shrimp Virus Peer Review and Risk Assessment Workshop, sponsored by the U.S. Environmental Protection Agency (EPA) in cooperation with the Joint Subcommittee on Aquaculture (JSA; National Science and Technology Council), held January 7–8, 1998, in Arlington, VA. The goals of the workshop were to:

- Complete a qualitative assessment of the risks associated with shrimp viruses, following the general risk assessment process developed by the Aquatic Nuisance Species Task Force.
- Evaluate the need for a future, more comprehensive risk assessment.
- Identify critical risk-relevant research needs.

The workshop focused on the scientific and technical aspects of the likelihood that nonindigenous viruses will become established in wild shrimp populations in the Gulf of Mexico and southeastern Atlantic coastal regions and on the potential ecological consequences of establishment. The workshop included 22 experts with varied backgrounds, including shrimp biology, toxicology, virology, marine ecology, ecological risk assessment, and shrimp aquaculture and processing. Before the workshop, participants received several background documents (ERG, 1997; JSA, 1997; ANSTF, 1996 [Appendix G]) and they were asked to prepare written premeeting comments for review by all participants. (These comments appear in Appendix C.) At the workshop, participants were divided into three groups, each of which was charged with evaluating the risks associated with one of the following categories of viral pathways:

- Aquaculture
- Shrimp processing
- Other potential sources

The risk that shrimp viruses pose to shrimp aquaculture operations was not considered as part of the scope of the workshop due to the limited time available; however, workshop participants believed that the risks to shrimp aquaculture should be given special attention as part of a subsequent technical or management workshop.

The qualitative risk assessment was conducted using the modified Aquatic Nuisance Species Task Force risk assessment approach (ANSTF, 1996; Appendix G). In developing the qualitative risk assessment, participants considered the following:

- Likelihood of viruses being present in the pathway
- Ability of the viruses to survive transit in the pathway
- Colonization potential of the viruses in native shrimp
- Spread potential of the virus within native shrimp populations
- Consequences of establishment

In general, workshop participants agreed that viruses could be associated with pathways leading to coastal environments and that they could survive in these pathways. Participants concluded that there is potential for viruses to colonize native shrimp in localized areas, such as an estuary or embayment, near the point of entry into the marine system. Some participants also noted that repeated viral introductions to an area will increase the risk of colonization.

Participants had widely divergent views on the potential for viruses to spread beyond the initial local area of colonization. This divergence largely reflects the high uncertainty associated with this aspect of exposure. Participants considered the potential for localized colonization and subsequent spread to be a critical aspect of evaluating the potential establishment of viruses in native shrimp.

Workshop participants discussed the impact that virus establishment could have on local shrimp populations (e.g., within an individual estuary). The participants determined that initial kill rates might be high but that the population would be likely to recover rapidly due to reintroduction of shrimp from other locales or compensatory increases in reproduction. Workshop participants concluded that the risk from viral introductions to the entire population of native shrimp along the southeastern Atlantic coast and within the Gulf of Mexico is relatively low, although there is a high degree of uncertainty associated with this evaluation.

The ability of workshop participants to address broader ecological risks in a comprehensive manner was limited by the time and information available. However, some participants thought that the issue of broader ecological risks is important and merits further consideration.

Workshop participants identified areas where further research and information would improve the assessment of risks and could help evaluate current conditions. They also identified actions for reducing uncertainty that should be given the highest priority, including:

- Improved diagnostic methods

- Surveys of wild shrimp populations for presence of the four nonindigenous viruses and for genetic composition

- Experiments to reduce uncertainties surrounding virus transmission and virulence

- Field epidemiologic studies

Participants identified other areas where additional research is needed to improve the ability to estimate risks to wild shrimp populations, including:

- Viral persistence
- Compensatory mechanisms
- Monitoring of imported shrimp
- Development of suitable population models
- Targeted surveys of nonpenaeid species to determine if they are susceptible to or carriers of nonindigenous viruses

Workshop participants believed that, given the existing knowledge base, it is currently not feasible to conduct a more comprehensive, quantitative assessment of the risks associated with nonindigenous shrimp viruses. Participants generally agreed that, at present, qualitative evaluations could be made, but they noted there is a great deal of uncertainty surrounding many key areas of the shrimp virus problem. Participants determined that there is a need to continue efforts to gather available data on shrimp virus effects and a need to conduct a systematic research effort that could be used to reduce the uncertainty of any subsequent risk assessments.

Workshop participants identified the following areas of concern where additional efforts should be focused:

- Management implications of shrimp viruses
- Risks of shrimp viruses to aquaculture operations
- Risks of shrimp viruses to nonpenaeid species

2. INTRODUCTION

This report highlights issues and conclusions from the Shrimp Virus Peer Review and Assessment workshop sponsored by the U.S. Environmental Protection Agency (EPA) in cooperation with the Joint Subcommittee on Aquaculture (JSA), held January 7–8, 1998, in Arlington, VA. The goals of the workshop were to:

- Complete a qualitative assessment of the risks associated with shrimp viruses, following the general risk assessment process developed by the Aquatic Nuisance Species Task Force (ANSTF)
- Evaluate the need for a future, more comprehensive risk assessment
- Identify critical risk-relevant research needs

The workshop focused on the scientific and technical aspects of the likelihood that nonindigenous viruses will become established in wild shrimp populations in the Gulf of Mexico and southeastern Atlantic coastal regions and on the potential consequences of such establishment.

This section provides an overview of the recently published JSA report (JSA, 1997) that formed the basis for the workshop, a description of the workshop process, and a discussion of the qualitative risk assessment approach used at the workshop. Section 2 of this document summarizes discussions held during the workshop on several aspects of the qualitative risk assessment process, and it contains a risk characterization developed by the workshop chair and breakout group chairs following the workshop's conclusion. Section 3 discusses actions for reducing uncertainty that were identified by participants during the workshop. The reports of each breakout group are contained in Appendix A.

2.1. JSA REPORT OVERVIEW

Dr. Kay Austin of EPA's National Center for Environmental Assessment, and a member of the JSA Shrimp Virus Work Group, discussed the work group's efforts to date and described events leading to the workshop. She provided an overview of the purpose, scope, and findings of the work group's report, entitled "An Evaluation of Potential Shrimp Virus Impacts on Cultured Shrimp and on Wild Shrimp Populations in the Gulf of Mexico and Southeastern U.S. Atlantic Coastal Waters" (hereinafter called JSA report) (JSA, 1997). Highlights of her presentation follow.

New, highly virulent viruses have been documented in foreign shrimp aquaculture. Consumer demand for shrimp continues to grow, and to meet this demand, the United States has greatly increased shrimp importation from areas of the world where pathogenic shrimp viruses are known to be endemic. Recent events have prompted calls for investigation into the actual risks to U.S. domestic resources. These events have included catastrophic viral outbreaks in shrimp aquaculture both in the United States and abroad, recent appearances of these organisms in shrimp in commercial retail stocks, and new information on the susceptibility of shrimp and other crustaceans to these organisms. While some of these viruses have severe and lethal effects in crowded aquaculture conditions, they are not known to pose threats to human health.

The U.S. shrimp industry (harvesting and processing alone) is valued at $3 billion per year. Imported shrimp account for more than 80% of the market. In 1995, imports exceeded domestic production by a ratio of four to one, amounting to 720 million pounds (in tails). The largest share of these imports comes from Latin America and Asia, areas of the world where shrimp viruses are endemic. Domestic aquaculture operations, in contrast, account for a much smaller portion of the U.S. market, ranging from 2 million pounds in 1991 to 4 million pounds in 1994.

The JSA, which is under the auspices of the President's National Science and Technology Council, formed the interagency Shrimp Virus Work Group in March 1996 to assess the risks associated with these emerging viral pathogens. Four Federal agencies are represented in the work group: the National Marine Fisheries Service (NMFS), EPA, the U.S. Fish and Wildlife Service (USFWS), and the U.S. Animal and Plant Health Inspection Service (APHIS). JSA charged the work group with developing a Federal interagency strategy to address the shrimp virus issue and to identify relevant research on viral stressors, their potential mode of transmission, and their potential for introduction to U.S. shrimp resources.

The work group recognized that the shrimp virus problem presents some unique issues in risk assessment. Members determined that the problem is a complex one that moves beyond the traditional single-chemical, single-species assessment process. The shrimp virus problem involves potentially nonindigenous viral stressors and has great potential to significantly impact the U.S. shrimp industry and other ecological components of coastal systems.

During its initial evaluation of the problem, the work group decided to base its approach on EPA's ecological risk assessment guidelines, which were published in draft form in 1996 (U.S. EPA, 1996). Because the work group determined that not enough information was available to complete an actual risk assessment, it followed a problem formulation approach that enabled the work group to summarize risk-relevant information available prior to January 1997 and to identify data gaps and critical research needs.

During its problem formulation activities, the work group developed a proposed management goal and identified potential viral sources, potential viral and other environmental stressors, and potential ecological effects. The work group also reached consensus on assessment endpoints and developed a conceptual model (Figure 1) that illustrates the linkages between human activities, viral stressors, and assessment endpoints of concern. The work group's report was completed in June 1997.

Significant findings of the JSA report include the following:

- Viral disease has been associated with severe declines in wild shrimp harvests in the Gulf of California. Populations of the blue shrimp, *Penaeus stylirostris*, and other less dominant species plummeted coincident with the observed occurrence of IHHNV disease in wild shrimp populations in the Gulf of California. The work group found that this is the best piece of epidemiologic information suggesting a link between introduced viruses and declines in wild shrimp populations. There remains considerable debate, however, regarding the validity of this association of disease and effects.

- Nonindigenous shrimp viruses have not been documented in wild U.S. shrimp populations; until recently, detection efforts have been minimal. Sampling techniques may have been inadequate, and the correct technology may not have been available to adequately detect the viruses.

- Numerous nonindigenous viral disease outbreaks have occurred in U.S. shrimp aquaculture since 1994, and frozen shrimp in commerce have been found to be contaminated with these viruses. Laboratory studies show that all life stages of shrimp are potentially at risk from at least one of the four viruses covered in the JSA 1997 report.

- Harvesting practices in foreign aquaculture could put U.S. domestic shrimp populations at risk. The work group learned that when an outbreak occurs in some foreign aquaculture operations, the affected crop is often harvested immediately and exported to avoid severe crop and monetary losses.

- Shrimp may be contaminated from a number of possible sources. The work group identified aquaculture and shrimp processing as two potentially important sources that may affect wild shrimp populations. Potential pathways for nonindigenous viruses to reach shrimp populations via these sources are shown in Figures 2 and 3. The work group also considered a number of other possible sources, such as live and frozen bait shrimp, ballast water, and natural spread by mechanisms such as hurricanes, floods, or animals. Research and display facilities also may be a source of exposure to wild populations.

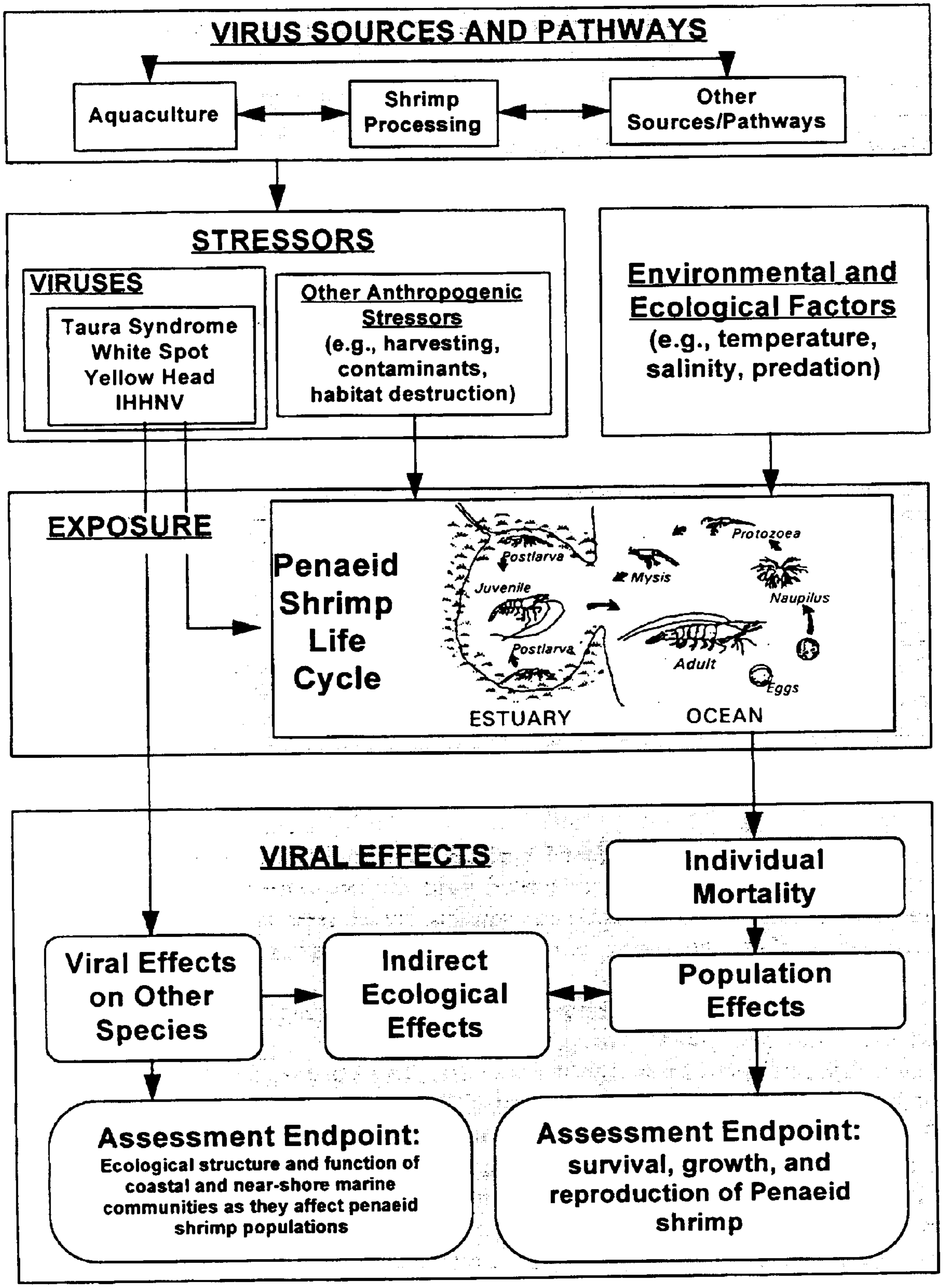


Figure 1. Shrimp virus conceptual model. This model was provided to workshop participants to assist with their discussions. Participants focused their discussions on viral stressors and direct effects on penaeid shrimp.

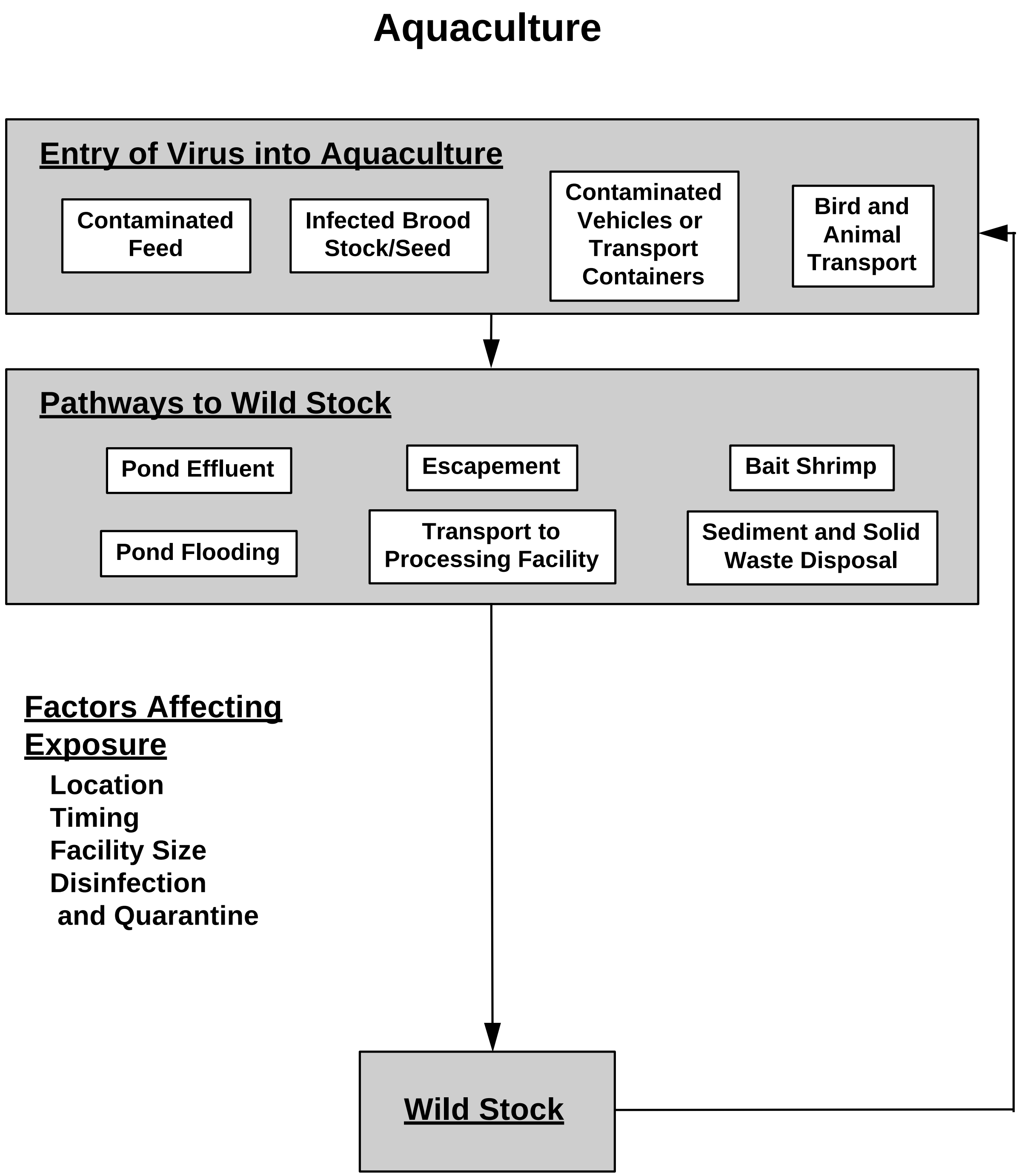


Figure 2. Conceptual model: virus sources and pathways for aquaculture.

Source: JSA, 1997.

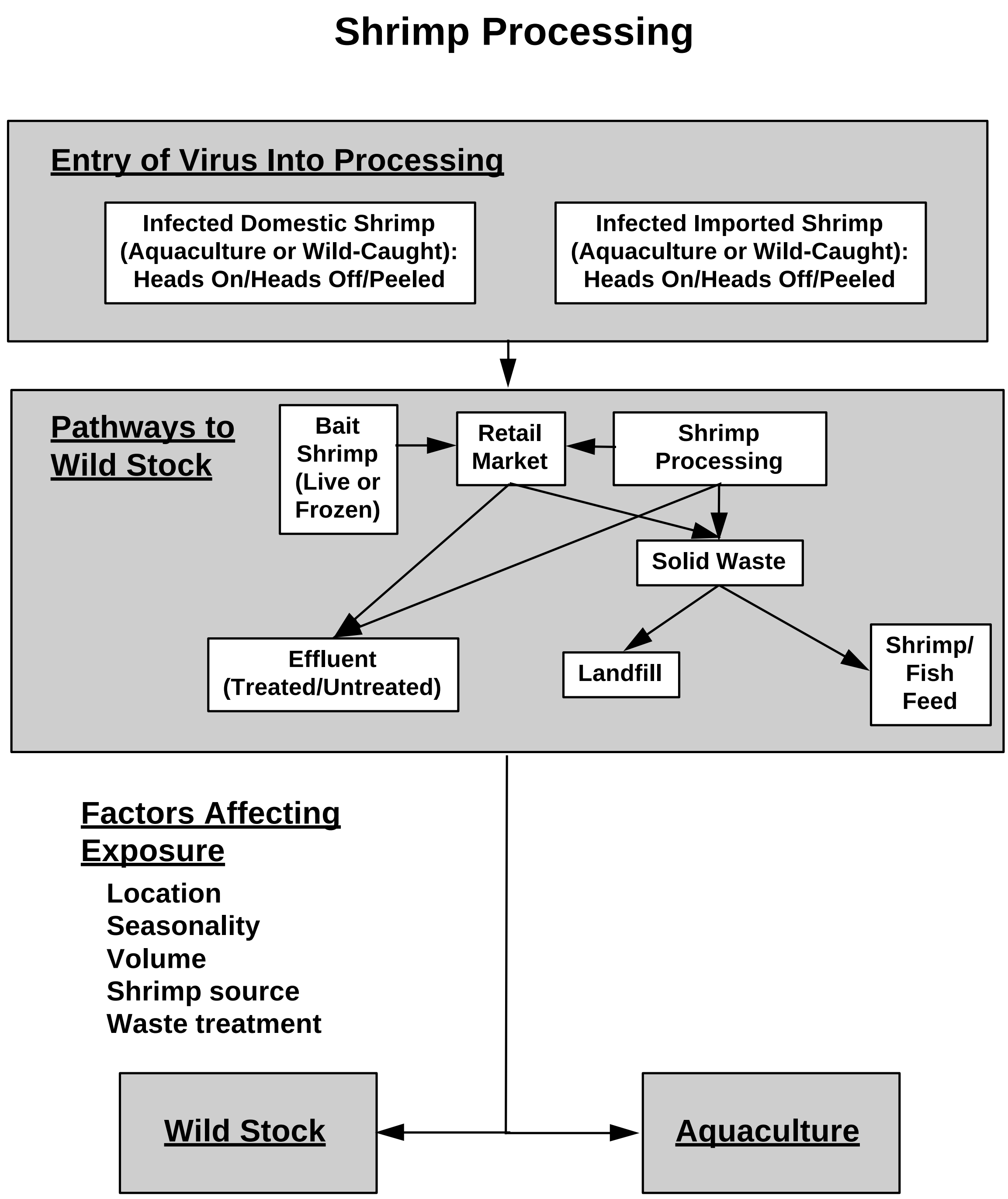


Figure 3. Conceptual model: virus sources and pathways for shrimp processing.
Source: JSA, 1997.

- Species other than shrimp may be at risk from these viruses. Viral disease could result in alterations to ecosystem structure or function, potentially affecting a wide range of endpoints, such as predator-prey relationships, competition, and nutrient cycling. Many other economically and ecologically important organisms that occupy coastal areas feed on juvenile shrimp, and impacts to these organisms could be serious if the wild shrimp populations on which they feed decline. Other organisms may be susceptible to disease themselves or serve as carriers of these viruses.

During July 1997, JSA and EPA sponsored public meetings in Charleston, SC; Mobile, AL; Brownsville, TX; and Thibodaux, LA, to gather stakeholder input on the shrimp virus issue and the JSA report. Stakeholders included individuals from the wild shrimp fishery industry, the shrimp aquaculture industry, the shrimp processing industry, environmental organizations, regulatory and resource management agencies, and the general public. The minutes of these stakeholder meetings were published in October 1997 (ERG, 1997).

2.2. PEER REVIEW OF JSA SHRIMP VIRUS REPORT

Prior to the workshop, Eastern Research Group (ERG) provided all experts with a number of documents and materials to help the experts prepare for the workshop and to assist them in developing a peer review of the JSA report. The materials provided included the JSA report (JSA, 1997), the minutes of the stakeholder meetings about the JSA report (ERG, 1997), and a copy of a qualitative risk assessment process for nonindigenous organisms (ANSTF, 1996). Panel members (Appendix B) were asked to review the material and prepare written comments to address questions on the following topics:

- Management goals, assessment endpoints, and the conceptual model
- Viral stressors and factors regulating shrimp populations
- Viral pathways and sources
- Stressor effects
- Comprehensive risk assessment and research needs

(Lists of the peer review experts and their breakout group discussion assignments are contained in Appendix B. The charge to experts and the experts' premeeting written comments are contained in Appendix C. Overheads prepared by the chairpersons that summarize the premeeting comments are contained in Appendix F.)

Below are summarized the peer review comments by expert panel members on aspects of the JSA report (excluding sections not directly relevant to the JSA report) important to developing a qualitative ecological risk assessment. The recommended modifications to information included in the JSA report were considered in discussions at the workshop and incorporated as appropriate into the qualitative risk assessment.

2.2.1. Management Goal, Assessment Endpoints, Conceptual Model, and Scope of the Assessment

The participants were charged with commenting on how well the JSA report's proposed management goal, assessment endpoints, and conceptual model reflected the dimensions of the shrimp virus problem. Those persons responding generally agreed that the proposed management goal adequately reflected the broader dimensions of the shrimp virus problem. However, a number of participants offered suggestions to further broaden the focus of the management goal to include the following: enlarging the report's geographic coverage to include the U.S. Pacific coast; focusing on risks to aquaculture; considering other potential pathogens (e.g., other viruses, bacteria, fungi) and other potentially susceptible organisms; addressing other environmental stressors potentially impacting wild shrimp populations (e.g., pollutants, coastal development); and evaluating the economic impacts related to developing (or not) alternative seafood production methods. One reviewer also indicated that the management goal may have been appropriate in 1996, as it assumed the four viruses were "new" and none had been established in U.S. coastal waters. However, the reviewer noted that there is growing evidence that at least one of the four viruses (WSSV) may have already become established. Other reviewers emphasized the need to keep the focus of the management goal and assessment endpoints narrow for the risk assessment to be manageable.

Participants generally agreed that the proposed assessment endpoints were adequate to address the scope of the problem. Many reviewers broadly interpreted or accepted the intent of the first assessment endpoint—"survival, reproduction of wild shrimp"—but they viewed the second proposed assessment endpoint—"ecological structure and function"—as too broad to be measurable or meaningful. Some believed that the second proposed endpoint should be deleted and replaced with one to address concerns for potential risks to a broad range of other marine organisms. A number of other recommendations were made to modify the proposed endpoints, including the following: emphasize risks of viruses to aquaculture, focus on ecological aspects not necessarily related to wild shrimp populations and harvest, and develop comprehensive data on the genetic structure and prevalence of viruses in natural populations. One expert expressed concern that the report did not focus enough on ecologically important, nonpenaeid species such as the grass shrimp.

Reviewers expressed a variety of opinions about the scope of the conceptual model and the risk assessment. While some experts indicated that the proposed conceptual model should be expanded to include the full range of probable risks and to develop a suite of related assessment endpoints, others emphasized that such an expansion would be overly ambitious for the initial phase of the risk assessment. It was recommended that all potentially important interactions should be identified in the conceptual model and connectivity between various endpoints or systems should be represented. One reviewer noted that the assessment might be expanded following the findings of the initial phase of the risk assessment.

2.2.2. Viral Stressors and Factors Regulating Shrimp Populations (Relevance of Laboratory Data, Human Health Concerns, Reliability of Available Identification Techniques)

Because of the lack of extensive field data on virus effects on wild shrimp populations, participants were asked to judge the relevancy of information on virus infectivity and effects from laboratory or intensive aquaculture to determine virus effects on wild shrimp populations. Opinions varied widely. Some participants viewed such information as totally irrelevant given conditions in an aquaculture setting, such as high densities that can potentially contribute to susceptibility, virus infectivity, and spread. Others noted that while this type of information could not be used to make reliable predictions about virus effects on wild populations, it is valuable in determining the potential for effects to occur in the wild. Reviewers noted that laboratory studies can be used to establish potential host range, and dose response data can be used to predict impacts to wild populations. In some cases, participants noted that such data can provide the best and most reliable information available.

The JSA report indicated that concerns for human health effects of shrimp viruses could be “ruled out” based on expert opinion and numerous observations, given the tremendous quantities of shrimp imports over the past 30 years and the lack of evidence of human health effects as a result of shrimp importation, processing, and consumption. Workshop participants were asked to comment on the report’s conclusions. Opinions varied; some experts were more cautious and expressed the opinion that it was premature to completely rule out human health effects, while others with perhaps more extensive experience working with the viruses of interest offered a differing point of view. The latter group noted that while one can never be absolutely certain that a nonhuman host virus will not become infectious to humans, it is highly unlikely that shrimp viruses will affect human health. Participants noted that viruses co-evolve with their hosts and become highly adapted to particular hosts. Given the tremendous evolutionary distance between vertebrates and invertebrates, it is improbable that these viruses could infect humans or other vertebrates, even by mutating. However, factual evidence to clarify this uncertainty is lacking.

The JSA report expressed concern about the availability and reliability of methods for isolation and identification of shrimp viruses in wild populations and environmental media. Expert panel members were asked to comment on their understanding of this issue. Opinions were divided; some panel members responded that identification techniques are reliable and effective, while others pointed out that detection methods have yielded mixed results. It was noted that bioassays, histologic examination, and serologic methods have been applied, but their specificity and sensitivity have been difficult to assess. Panelists said that methods are available for only three or four of the viruses focused on by the JSA report, and the complex nature of this testing may not allow definitive conclusions to be made about occurrence. Most participants commented that additional research is needed to develop molecular, immunologic, and diagnostic techniques to screen for viruses in wild shrimp tissues, feed, and environmental media potentially contaminated by shrimp viruses. Given current technology, one expert noted that it is impossible to determine with certainty virus occurrence in large volumes of soil or water.

2.2.3. Viral Pathways and Sources

The JSA Shrimp Virus Work Group considered aquaculture and shrimp processing to be primary pathways of concern leading to exposure of pathogenic shrimp viruses to wild shrimp populations, and other sources were identified as secondary pathways for exposure. Workshop participants were asked to judge the acceptability of these sources as potential pathways for virus introduction to wild shrimp populations.

2.2.3.1. *Aquaculture*

One common theme expressed by participants was that there is little scientific evidence to either confirm or refute the occurrence of epizootics among wild shrimp associated with naturally occurring or introduced viruses. Participants noted that evidence indicates that one shrimp virus may be present in wild populations in U.S. coastal waters, but its source is unknown. One expert remarked that no convincing causal relationship has been established between outbreaks of virus in aquaculture facilities and virus transmission to wild stocks. The expert continued that although no direct link has been established, it does not rule out that it has occurred previously or will occur in the future. The expert added that, to date, wild populations have not been adequately monitored. He concluded that when monitoring is available, we may be able to track the movement of virus infection, eventually resolving this issue. One other reviewer also noted that without simultaneously isolating viruses from aquaculture and a geographically located shrimp population (possible with the development of gene probes), the role of aquaculture in infecting wild shrimp remains speculative. Participants emphasized that resolving this issue is highly problematic and a critical element to the risk assessment.

2.2.3.2. *Shrimp Processing*

The JSA report suggests that shrimp processing could be another primary source for introducing pathogenic shrimp viruses to U.S. coastal waters. Workshop participants were asked to consider how evidence from wild shrimp populations either supported or refuted the importance of shrimp processing as a potential source for shrimp virus. As with shrimp aquaculture, in general, panel members concluded that there was little scientific evidence to suggest a strong link between processed shrimp or shrimp process wastes and the occurrence of shrimp viruses in wild shrimp populations. However, several experts noted that disposal of wash water from shrimp processing facilities directly to receiving waters that support any phase of wild shrimp development should continue to be a concern. One theme reflected in reviewer comments was concern that the practice of some producers to harvest diseased shrimp for export makes this one of the more likely potential sources of virus contamination of wild shrimp populations (because the United States imports significant quantities of shrimp). One other reviewer concluded that even though shrimp processing could introduce virus, there is not enough known about viral persistence in nature to determine whether shrimp processing represents a realistic source of pathogenic shrimp virus introduction to wild shrimp.

2.2.3.3. *Other Potential Sources*

Workshop participants were also asked to consider the potential role of sources other than aquaculture and shrimp processing (secondary sources) in introducing pathogenic shrimp virus to wild shrimp. Those panel members responding considered bird feces and ballast water transfer as likely other potential sources. Natural spread, bait shrimp, and the introduction of secondary hosts were considered to be less important "other" potential sources. It was generally agreed that while those other sources suggested by participants and those listed in the JSA report could be plausible, their relative contribution was unknown. Participants expressed concern that management of these other potential sources could be problematic. However, one dissenting panel member expressed the opinion that an evaluation of the existing data with respect to the probabilities of transmission and establishment should be done for all potential sources.

The JSA report expressed concern that manufactured shrimp feed could be a potential other source of shrimp virus. Participants were asked to consider the importance of shrimp feed as a potential source of pathogenic shrimp viruses. Panel members were divided in their responses to this question. Many expressed the opinion that processing temperatures were adequate to eliminate shrimp feed as a source of shrimp virus, while others noted that temperatures may not be adequate and shrimp feed should continue to be of concern as a possible other source. However, one expert emphasized that the likelihood of shrimp feed as a source could be determined only by knowledge of virulence of the specific type of virus, its viability, and the length of time materials were held at temperatures, especially those processed at lower (70°C) temperatures. To further eliminate shrimp feed as a potential other source, one participant noted that farms should be discouraged from using or supplementing manufactured food with natural feeds.

2.2.4. Stressor Effects

Participants were asked to consider how the available evidence regarding the effect of introduced shrimp virus on wild shrimp populations should be interpreted. In general, workshop participants indicated that there is little convincing information or scientific data on effects of introduced pathogenic shrimp viruses on wild shrimp populations. Some participants believed the available information, when considered carefully, could be useful in identifying underlying problems. One expert cautioned that available evidence should be considered individually for each virus and host system. Another expert noted that while there is clear evidence that viruses have been introduced to aquaculture, it is not known how these may relate to the observed declines such as those observed in the Gulf of California example considered by the JSA report. Reviewers also noted that such associations of virus occurrence cannot be made without considering the role of other important environmental factors in wild shrimp population declines, notably overfishing, El Nino, pollution, and environmental degradation. Reviewers indicated that there is a critical need for research to address this issue.

The JSA report discussed the importance of virus effects on nonshrimp species, and panel members were asked to comment on this issue. For the most part, participants agreed that potential virus effects on nonshrimp species are generally unknown but of significant concern. One respondent observed that it is well documented that some viruses can infect other crustacean species, noting that WSSV has been detected by polymerase chain reaction (PCR) in both cultured and wild shrimp, prawns, crabs, and other arthropods in different Asian countries. He concluded that the potential threat to U.S. shrimp, nonshrimp, and the ecosystem as a whole could not be ruled out. However, one member noted that while nonshrimp species are important ecologically, pathogenicity of viruses is usually species specific. In contrast, another participant commenting on the JSA report's discussion of effects on nonshrimp species thought that these effects should be considered not very great, and he pointed out that the report also failed to emphasize concern for effects on nonpenaeid shrimp species, such as the grass shrimp. This concern stemmed from the knowledge that other parasites of shrimp can be harmful to other marine organisms and that these pathogenic shrimp viruses could cause serious impacts to sport commercial fisheries by reducing available food sources such as the grass shrimp. Yet another expert noted that effects on nonshrimp species should be considered important, especially on susceptible species with low population levels. One participant felt this was an extremely important issue but noted it is probably difficult to evaluate on the short term.

2.3. SHRIMP VIRUS PEER REVIEW AND RISK ASSESSMENT WORKSHOP PROCESS

At the beginning of the workshop, the workshop chairperson, Dr. Charles Menzie (Menzie-Cura & Associates) reviewed the agenda (included in this report as Appendix D), explained the workshop's format, and reviewed the workshop's goals, which were to:

- Complete a qualitative assessment of the risks associated with nonindigenous shrimp viruses, following the general risk assessment process developed by the ANSTF
- Evaluate the need for a future, more comprehensive risk assessment
- Identify critical risk-relevant research needs

Dr. Menzie explained that the workshop report would be used to provide information for a proposed workshop to identify potential risk management options. The proposed workshop, sponsored by JSA and NMFS, was held in July 1998. Peer review experts were divided into three breakout groups, each of which was charged with evaluating the risks associated with one of three viral pathways (aquaculture, shrimp processing, and other potential sources).

Three experts in ecological risk assessment were selected as breakout group leaders: Dr. Wayne Munns (EPA Office of Research and Development), who facilitated discussions on aquaculture; Dr. John Gentile (University of Miami), who facilitated discussions on shrimp processing; and Dr. Anne Fairbrother (Ecological Planning and Toxicology, Inc.), who facilitated discussions on other potential sources. (See Appendix B for breakout group assignments.) After the workshop, Dr. Munns prepared the report of the aquaculture breakout group (Appendix A-1), Dr. Gentile prepared the report of the shrimp processing breakout group (Appendix A-2), Dr.

Fairbrother prepared the report of the other pathways breakout group (Appendix A-3), and Dr. Menzie prepared the qualitative risk assessment (Section 3). Workshop participants were asked to review and comment on the breakout group reports prior to preparation of this final document.

2.4. QUALITATIVE RISK ASSESSMENT METHODOLOGY

Mr. Richard Orr, of the U.S. Department of Agriculture, Animal, and Plant Health Inspection Services (USDA-APHIS), provided participants with an overview of the qualitative risk assessment methodology to be used at the workshop. The process was based on the ANSTF risk assessment approach (ANSTF, 1996), which provides a qualitative assessment of the probability and consequences of establishment of a nonindigenous species in a new environment. (A copy of the ANSTF report [1996] is contained in Appendix G.) Mr. Orr noted that the methodology may be used as a subjective evaluation, or it may be quantified to the extent possible depending on the needs of the analysis. He reviewed an assessment on black carp to illustrate the application of this process to a nonindigenous species. Both documents were provided to workshop experts as background information prior to the workshop.

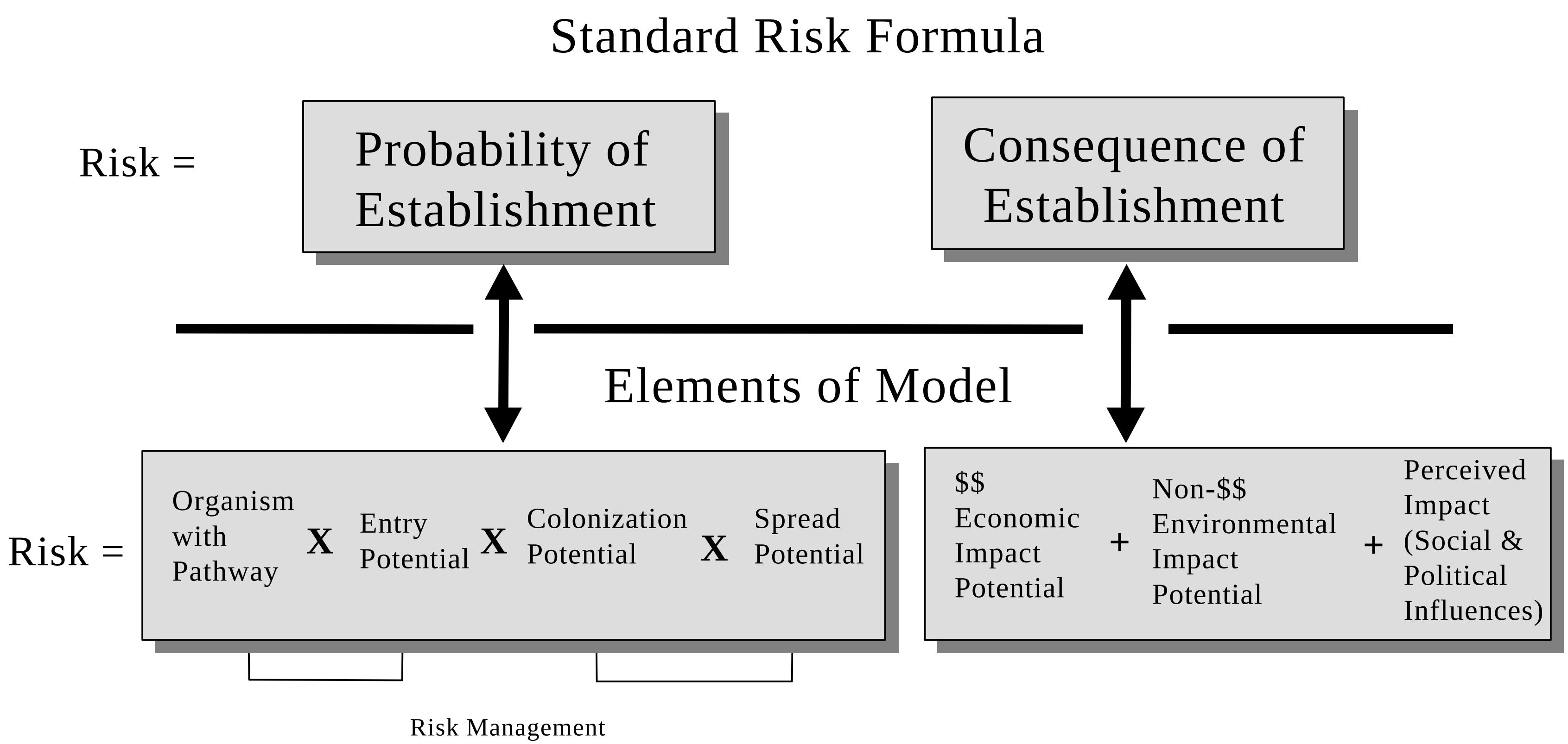
Mr. Orr explained that the risk assessment model is divided into two major components: the "probability of establishment" and the "consequences of establishment" (see Figure 4, which contains the risk assessment model from the Report to the Aquatic Nuisance Species Task Force). These components of the model are further divided into basic elements that serve to focus scientific, technical, and other relevant information for the assessment. Mr. Orr discussed how the following elements could be used to estimate the probability of establishment of viral pathogens in wild shrimp populations:

- Probability of the nonindigenous organism being on, with, or in the pathway
- Probability of the organism surviving in transit
- Probability of the organism successfully colonizing and maintaining a population where introduced
- Probability of the organism spreading beyond the colonized area

The following elements are used in the ANSTF approach to evaluate the consequence of establishment of a nonindigenous species (see, Appendix G, p. 22):

- Economic impact
- Environmental impact

Risk Assessment Model



- For model simplification, the various elements are depicted as being independent of one another
- The order of the elements in the model does not necessarily reflect the order of calculation

Figure 4. Risk Assessment Model from the Report to the Aquatic Nuisance Species Task Force

Source: Adapted from RAM, 1996.

- Impact from social and/or political influences

For the purposes of the Shrimp Virus Peer Review and Risk Assessment Workshop, only environmental impacts were evaluated. It was recommended that economic and perceived impacts of establishment be considered at a workshop on risk management options, which was held in July 1998.

Mr. Orr stressed that it is critical for the qualitative risk assessment to capture and communicate the uncertainty that surrounds the available information about shrimp viruses.

3. QUALITATIVE RISK ASSESSMENT

3.1. THE RISK ASSESSMENT PROCESS

Workshop participants began the risk assessment process by reviewing the management goal and assessment endpoints presented in the JSA report (JSA, 1997). Participants evaluated the risks associated with aquaculture, shrimp processing, or other potential sources. In the breakout groups, participants considered the ecological risks associated with each identified viral pathway. The evaluation of each pathway was conducted independently. It is important to note that participants did not attempt to rank the relative risk of the three identified sources.

Each breakout group evaluated both the potential for establishment of the viruses via the identified pathways and the potential ecological consequences of establishment. The breakout groups considered the four following elements of the potential for establishment of viruses via the identified pathways:

- Association of nonindigenous viruses with the pathway
- Entry of nonindigenous viruses into coastal waters via the pathway (including survival)
- Colonization/infection of shrimp at the local level
- Spread of nonindigenous viruses to the shrimp populations at large

To determine the probability of establishment of nonindigenous viruses, the breakout groups rated each of these elements as either low, medium, or high. The consequences of establishment were similarly rated. During their deliberations, the breakout groups were asked to identify the level of uncertainty (ranging from very uncertain to very certain) associated with each of the elements describing the potential for and consequences of establishment of nonindigenous pathogenic shrimp viruses.

Using the method set forth in the ANSTF (1996) report (Appendix G), workshop participants estimated the overall risk by compiling the risks associated with the individual elements of the process (i.e., [1] the four elements of the probability of establishment and [2] the consequence of establishment). The probability of establishment is determined by the lowest ranking of any of the four elements. For example, if elements under the probability of establishment had rankings of high, high, low, and medium, the overall probability of establishment would be considered low. This approach is reasonable because, for an organism to become established, each of the elements must occur. Assuming the elements are independent of each other, combining a series of probabilities will give a probability much lower than the individual element ratings. The conservatism of this approach is justified by the general high degree of biological uncertainty that is found throughout the process (ANSTF, 1996).

Rankings for the probability of establishment and the consequence of establishment are combined into an overall level of risk as shown in Table 1.

These rankings, which are based on expert judgment, should not be considered separately from the discussion and rationale provided by the workshop participants. As noted in the ANSTF (1996) report, "the strength of the Review Process is not in the element-rating but in the detailed biological and other relevant information statements that motivate them."

After evaluating the probability of establishment for their respective pathways and the consequences of establishment at the local and regional (e.g., Gulf of Mexico) population levels, the three breakout groups presented their findings in a plenary session. Breakout group findings are found in Appendices A-1, A-2, and A-3, while the main body of this document primarily reflects plenary discussions but incorporates breakout group findings when there was a lack of consensus. Following the conclusion of the expert workshop, the breakout group chairpersons and the workshop chairperson met to discuss the breakout group findings and their reports and to develop a risk characterization for the assessment using ANSTF methodologies.

Table 1. Combining the rankings for the probability of establishment and the consequences of establishment into an overall estimate of risk

If the overall probability of establishment is:	And the consequence of establishment is:	Then the overall risk ranking is:
High Medium Low	High High High	High High Medium
High Medium Low	Medium Medium Medium	High Medium Medium
High Medium Low	Low Low Low	Medium Medium Low

3.2. QUALITATIVE RISK ASSESSMENT RESULTS

This section summarizes discussions held during the workshop on several aspects of the risk assessment process:

- Management goal and assessment endpoints that frame the assessment (Section 3.2.1)
- The probability of establishment of shrimp viruses (Section 3.2.2)
- The consequences of establishment (Section 3.2.3)
- A characterization of the risks resulting from a combination of the probability and consequences of establishment (Section 3.2.4)

The reports of the three breakout groups are contained in Appendix A. Tables 2 through 5 provide the risk rankings assigned to various pathways by the breakout groups, which are summarized in Sections 3.2.2 through 3.2.4.

3.2.1. Management Goal and Assessment Endpoints

Workshop participants were asked to evaluate the completeness and adequacy of both the management goal and the assessment endpoints identified in the JSA report (JSA, 1997). In the ecological risk assessment process, the management goal is intended to reflect the management context of the assessment, while the assessment endpoints are explicit expressions of the environmental values to be protected, which serve as the focal points for an assessment.

The management goal identified in the JSA report is to:

- Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. coastal waters while minimizing possible impacts on shrimp importation, processing, and aquaculture operations.

A number of participants thought that the management goal should be broadened to include risks to aquaculture operations. Participants concurred that these risks are important, but because of the limited time available for workshop discussions, they agreed that risks to aquaculture operations would not be considered during the workshop. Participants recommended instead that risks to shrimp in aquaculture operations and management of those risks be the subject of a separate workshop.

Table 2. Summary of aquaculture breakout group risk rankings

Refer to supporting discussion in the text to properly evaluate information presented in this table. The risk assessment process is described in Section 3.1 and Appendix G.

	Pathways to the environment				
Probability of establishment	**Escapement**	**Pond flooding**	**Pond effluent**	**Transport to processing facility**	**Sediment and solid waste disposal**
Association with pathway	High/very certain	High/very certain	High/very certain	High/very certain	High/very certain
Entry potential	High/very certain or low/reasonably certain[a]	Low/ very certain	Medium/very certain	Low/ reasonably certain	Low/ reasonably certain
Colonization potential	Low (or medium to high)[b]/very certain	Low (or medium to high)[b]/very certain	Low (or medium to high)[b]/very certain	Low (or medium to high)[b]/very certain	Low (or medium to high)[b]/very certain
Spread potential	Low/relatively uncertain to high/very uncertain[c]	Low/relatively uncertain to high/very uncertain[c]	Low/relatively uncertain to high/very uncertain[c]	Low/relatively uncertain to high/very uncertain[c]	Low/relatively uncertain to high/very uncertain[c]
Overall probability of establishment	Low to high	Low	Low to medium	Low	Low
Consequences of establishment	Low to medium/very uncertain	Low to medium/very uncertain	Low to medium/very uncertain	Low to medium/very uncertain	Low to medium/very uncertain
Overall risk estimate	Low to high	Low to medium	Low to medium	Low to medium	Low to medium

[a]High if pond is infected and shrimp escape from pond; low otherwise.

[b]Some breakout group members believed that the potential was medium and would be high if the aquaculture industry expands significantly along the Gulf Coast.

[c]The breakout group could not reach consensus; opinions on entry potential ranged from low to high.

Table 3. Summary of shrimp processing breakout group risk rankings

Refer to supporting discussion in the text to properly evaluate information presented in this table. The risk assessment process is described in Section 3.1 and Appendix G.

Probability of establishment		**Pathways to the environment**			
		Treated effluent	**Untreated effluent**	**Landfill**	**Shrimp/fish feeds**
Association with pathway		High/ very certain	High/ very certain	High/ very certain	High/very certain
Entry potential		Low/very certain	High/very certain	Medium/ reasonably certain	Low/ very certain
Colonization potential		Low/ very certain	Medium/ moderately certain	Low/ reasonably uncertain	Low/ very certain
Spread potential		Low/ very certain	Medium/ moderately certain	Low/ reasonably uncertain	Low/ very certain
Overall probability of establishment		**Low**	**Medium**	**Low**	**Low**
Consequences of establishment	**Local**	Low-medium/ reasonably uncertain	Low-medium/ reasonably uncertain	Low-medium/ reasonably uncertain	Low-medium/ reasonably uncertain
	Large scale	Low/highly uncertain	Low/highly uncertain	Low/highly uncertain	Low/highly uncertain
Overall risk estimate	**Local**	Low-medium	Medium	Low-medium	Low-medium
	Large scale	Low	Medium	Low	Low

Table 4. Summary of other pathways breakout group risk rankings for likely pathways to the environment

Refer to supporting discussion in the text to properly evaluate information presented in this table. The risk assessment process is described in Section 3.1 and Appendix G.

Probability of establishment	Ballast water	Bait shrimp		Shrimp feed		Animal vectors
		Foreign	Domestic	No heat	Heat-treated	
Association with pathway	High/moderately certain	High/ moderately certain	Low/very certain	Medium/ moderately certain	Medium/ moderately certain	High/very or reasonably certain[a]
Entry potential	High/very certain	High/very certain	High/very certain	High/very certain	Low/very certain	High/reasonably certain
Colonization potential	Low/moderately certain	High/very uncertain	High/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium to high/ relatively uncertain
Spread potential	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain
Overall probability of establishment	Low	Medium	Low	Medium	Low	Medium

[a]Very certain for gulls and freshwater and marine invertebrates; reasonably certain for other vertebrates.

Table 5. Summary of other pathways breakout group risk rankings for secondary or incidental pathways to the environment

Refer to supporting discussion in the text to properly evaluate information presented in this table. These pathways were rated individually by breakout group members, and there was no group discussion of these ratings. Consequences of establishment were not rated for these pathways. The risk assessment process is described in Section 3.1 and Appendix G.

Probability of establishment	**Natural spread**	**Research and display facilities**	**Human sewage**	**Fishing vessels**	**Hobby and ornamental displays**	**Live seafood distribution**	**Other crustacean aquaculture**	**Incidental introductions**
Association with pathway	Medium/very uncertain	Low/ moderately certain to high/very certain	Medium/ very uncertain	Low to medium/ moderately certain	Low/ moderately certain	Low/ reasonably uncertain	Low/very uncertain to medium/ moderately certain	Low/very uncertain
Entry potential	High/very uncertain	High/ moderately to very certain	Medium/ very uncertain	High/ reasonably certain	High/ moderately certain	High/ moderately certain	Low/very uncertain to medium/ reasonably certain	Low/very uncertain
Colonization potential	High/very uncertain	Low/very certain to high/very uncertain	Medium/ very uncertain	Medium/ reasonably uncertain	Low/ moderately certain	Low/ reasonably uncertain	Low/very uncertain to medium/very uncertain	Low/very uncertain
Spread potential	High/very uncertain	Low/ relatively certain to high/very uncertain	Medium/ very uncertain	Medium/ very uncertain	Medium/very uncertain	Medium/very uncertain	Low/very uncertain to medium/very uncertain	Low/very uncertain
Overall probability of establishment	Medium	Low to medium	Medium	Low to medium	Low	Low	Low to medium	Low

The JSA report (1997) identifies two assessment endpoints:

- Survival, growth, and reproduction of wild penaeid shrimp populations in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters

- Ecological structure and function of coastal and near-shore marine communities as they affect wild penaeid shrimp populations

Workshop participants elected to focus their efforts on the first assessment endpoint (direct effects to wild shrimp populations) for the following reasons:

- Risks to wild shrimp populations are of primary concern

- Information on secondary effects is even more limited than information on direct effects on shrimp

- There was limited time available at the workshop for evaluating all possible direct and indirect effects.

Participants recognized the potential for direct effects on organisms other than penaeid shrimp and the potential for indirect effects; however, these effects were not discussed in detail during the workshop. They are, however, a potential concern for resource managers.

3.2.2. Probability of Establishment

This section summarizes breakout group discussions concerning the elements of the probability of establishment, which include association with pathway, entry potential, colonization (infection) potential, and spread potential.

Workshop participants recognized that differences among the four viruses could result in variations in the risk rankings associated with the elements comprising the probability of establishment for an individual virus. For example, if one virus were to survive longer than another virus in the marine environment, it could affect the entry potential ranking. However, the breakout groups decided to consider the potential for establishment of nonindigenous viruses as a single group but agreed to identify any unique differences that might alter risk rankings. A summary of the characteristics of the four viruses is contained in Table 6.

Table 6. Virus persistence, virulence, and infectivity

	IHHNV	TSV	YHV	WSSV
Persistence (1 = least, 4 = most)	**3.5**	**3.5**	**1.5**	**1.5**
Virulence to Gulf of Mexico species (1 = least, 4 = most)	1	2	3	4
	Relative infectivity			
Penaeus setiferus				
Larvae	—	—	ND	ND
Post-larvae	—	++	—	++
Juvenile	+	+	++	++
Adult	ND	+	ND	ND
Penaeus duorarum				
Larvae	—	—	ND	ND
Post-larvae	—	—	—	++
Juvenile	+	+	++	+
Adult	ND	ND	ND	ND
Penaeus aztecus				
Larvae	—	—	ND	ND
Post-larvae	—	+	—	++
Juvenile	+	+	++	+
Adult	ND	ND	ND	ND

Infectivity:

ND = No data
+ = Infectious
++ = Mortality
— = Tried but negative

3.2.2.1. *Association With the Pathway*

Breakout groups concluded with moderate to high certainty that there is a high likelihood that viruses are present in the aquaculture pathway, shrimp processing pathway, and some of the other potential pathways.

3.2.2.1.1. *Aquaculture*. The occurrence of nonindigenous viruses in U.S. aquaculture operations is well documented. As summarized in the JSA report, TSV has been identified in disease outbreaks in Hawaii, Texas, and South Carolina (Lightner, 1996a,b). IHHNV was first identified in Hawaii (Lightner et al., 1983a,b) and was subsequently observed in farms in South Carolina, Texas, and Florida (Fulks and Main, 1992). WSSV and YHV also have been documented at a shrimp farm in Texas (Lightner, 1996a,b). WSSV and YHV are considered to be of Asian origin; TSV and IHHNV are thought to have originated in Latin America. Workshop participants noted that the origins of these viruses are not always traceable to their ultimate sources, but it was suggested that their introduction to the United States may have resulted from the importation of infected shrimp from other regions of the world (e.g., Latin America and Asia).

3.2.2.1.2. *Shrimp processing*. Shrimp viruses can be brought into the United States with imported shrimp that are subsequently processed or used for other purposes (e.g., feed, bait shrimp, and retail sale). Of the shrimp processed in the United States, 80% of the total crop is foreign and 20% is domestic in origin. Pathogenic viruses have been identified in imported shrimp sold in this country. Breakout group members concluded with high certainty that the probability of association is high for all pathways considered (effluent, landfill, and shrimp and fish feed).

3.2.2.1.3. *Other pathways*. Other “primary” pathways described in the JSA report and considered by workshop participants include ballast water, bait shrimp, animal vectors, and shrimp feed. There appear to be no data on the occurrence of shrimp viruses in ballast water (or any of its components). Nonetheless, it is known that many organisms are discharged routinely with ballast water (including species of mysid shrimp, some of which have colonized bays and estuaries with devastating effects). There is, therefore, a high probability that ballast water could contain shrimp viruses, whether free living, attached to particulate matter, or in dead or infected shrimp.

Anglers use shrimp as bait when fishing in estuaries for fish that eat shrimp. They purchase bait from bait shops, or they use shrimp sold in grocery stores for human consumption. Bait shrimp generally are smaller than those sold for human consumption. They may originate from aquaculture facilities that have harvested their shrimp prior to full growout because of a viral outbreak. Some participants thought that Latin American and Asian producers may freeze these small shrimp and ship them to the United States for sale as bait, while the larger, uninfected shrimp will be sold at premium prices for human consumption. Therefore, there is a high probability that some imported bait shrimp may contain viruses.

Both live and frozen shrimp may be sold as bait. However, only native species of aquaculture shrimp may be harvested and sold as live bait. Some states (e.g., South Carolina) allow the use of nonnative farm shrimp as frozen bait. Native shrimp used in aquaculture are known to sometimes carry indigenous viruses (such as baculovirus, BP) but to date, there is no evidence that they carry nonindigenous viruses. Furthermore, any of these shrimp that are harvested early due to perceived disease problems are likely to be sold as frozen bait rather than as fresh bait. Therefore, there is a low probability that live shrimp used for bait will carry nonindigenous viruses.

Shrimp feed is made from soy protein, fish protein (including anchovies and menhaden), shrimp heads, and other types of shrimp and crustaceans (e.g., *Artemia*). Because the heads and other body parts of infected shrimp can carry a high concentration of viruses, workshop participants believed that there is a medium probability that the shrimp parts used as an ingredient in shrimp feed may be contaminated with the viruses. Although pathogenic nonindigenous viruses may be associated with this pathway, workshop participants concluded that the viruses are likely to be destroyed during processing of the shrimp feed (see Section 3.2.2.2).

Animal vectors such as gulls and freshwater and marine invertebrates were considered as another possible source for viral entry. For example, gulls and other scavengers, such as raccoons, are often seen feeding on dead shrimp and other organic matter associated with aquaculture facilities that have undergone viral outbreaks. Workshop participants believed there was a high probability of viral association with this pathway.

Workshop participants considered a number of other pathways to have a low to medium probability for viral association. Due to time limitations, these pathways, including natural spread of the viruses, research and display facilities, human sewage, fishing vessels, hobby and ornamental displays, live seafood distribution, other crustacean aquaculture, and incidental introductions, could not be discussed in detail at the workshop.

3.2.2.2. ***Entry Potential***

Entry potential includes the probability of viruses surviving in transit and the probability of their transport to coastal waters. Each breakout group recognized that the entry potential of nonindigenous viruses depends on the pathway of arrival. For example, the survival and entry characteristics of viruses found in shrimp processing effluents may be quite different from those found in ship ballast waters. In addition, the breakout groups recognized that entry potential depends on location. For example, viruses associated with shrimp that are raised, processed, or disposed of in locations far inland are less likely to reach coastal waters than are viruses that are associated with shrimp that are raised, processed, or disposed of along the coast. Workshop participants evaluated subpathways within each of the major pathway categories (aquaculture, shrimp processing, and other source pathways) and described entry potentials for viruses as ranging from low to high. Participants found the level of certainty associated with these evaluations to be quite variable.

3.2.2.2.1. ***Aquaculture*****.** The aquaculture breakout group considered the six subpathways from aquaculture to wild shrimp stocks identified in the conceptual model contained in the JSA report. Many breakout group members believed that the escapement subpathway (including both accidental and intentional releases, as well as "escape" via transport of shrimp tissue by the predatory activities of other animals) was the most likely route of release of viruses to the environment and that viruses were likely to survive when transported via this pathway. (As discussed in the following paragraphs, however, some breakout group members believed that the sediment and effluent pathways, which the group tabled because of a lack of crucial data, may also be important.)

The aquaculture breakout group noted that the entry potential via escapement (and other pathways) is likely to be related to the conditions in the pond (i.e., the presence and degree of infection by the viruses), the life stage of the shrimp (e.g., postlarvae may be more likely than adult shrimp to escape by passing through engineering controls), and the design of pond control systems. They concluded with relatively high certainty that the probability of surviving in transit would be high if conditions are favorable but assigned a low probability of survival if they are not.

The aquaculture breakout group had considerable discussion about the ability of viruses to survive in pond effluents and sediments. There is suggestive evidence about this potential pathway. TSV has been documented in water but not specifically in effluent waters. A workshop observer communicated results of an experiment that suggest that caged shrimp exposed in infected ponds developed disease (shrimp developed disease when exposed within 1 to 2 days to experimentally inoculated water, but they did not develop disease when exposed on days 3 to 5 following inoculation) (R. Laramore, personal communication, 1998). In 1995, HSF, Ltd., and the Arroyo Aquaculture Association conducted several trials in which cages were floated within a shrimp growout pond that had experienced a TSV epidemic and with pond water in tanks. The cages were suspended above the pond bottom and stocked with juvenile *P. vannamei.*

One participant noted that no TSV was detected in shrimp exposed for 30 days under these conditions. These results suggest that TSV may be transmitted during the acute but not the chronic stages of the disease. Other data suggest that IHHNV can survive in water in an infective state for at least 24 days (Glover et al., 1995). Another participant noted that viruses can spread quickly from pond to pond on aquaculture facilities, but it is not known how this transmission occurs. Based on this information, the aquaculture breakout group estimated that there is a medium potential that effluents released from infected farm ponds are a viable pathway for exposure to native populations; however, the breakout group was very uncertain about this estimate.

Pond flooding, sediment and solid waste disposal, and transport of shrimp to processing facilities were thought to have low likelihoods for entry potential, with uncertainties ranging from reasonably certain to very certain.

3.2.2.2.2. ***Shrimp processing.*** The shrimp processing breakout group identified two subpathways for which there is a medium to high potential for viruses to enter coastal areas: untreated effluents from shrimp processing facilities and solid wastes from disposal facilities near coastal areas that receive waste from shrimp processing facilities. The breakout group concluded with high certainty that there is a low potential for viable shrimp viruses to survive in effluents that are treated and disinfected at municipal facilities, and therefore, there is a low potential for entry of viable shrimp viruses to coastal areas from this pathway.

The shrimp processing breakout group estimated that approximately 50% of shrimp processing liquid effluent is untreated and that virus-contaminated discharges may therefore be released regularly into the environment. The breakout group was very certain that the probability of the organism surviving in transit—and therefore entering the environment through this pathway—is high.

Because of the uncertainties associated with the amounts of material reaching landfills, the types of vectors, and the threshold amount of virus required to infect the wild and aquaculture populations, the shrimp processing breakout group found it difficult to assess the probability of establishment of shrimp viruses from solid waste disposal facilities. Most breakout group members generally agreed that the shells, and particularly the heads, of foreign farmed and wild shrimp are highly likely to contain viruses. Considering these factors, breakout group participants concluded that these viruses are likely to persist for some time in landfill settings. Land crabs and seagulls are thought to be possible vectors for moving viruses from the landfills to estuarine waters. When these animals consume virus-contaminated materials, viruses might pass through their digestive systems in an infective state. The breakout group noted that TSV remains infective following gut passage in gulls, and the breakout group on other pathways suggested several other possible vectors for viral transmission. It is not known whether the concentrations and frequency of virus introduction from such vectors is sufficient to infect wild and aquaculture shrimp populations. The shrimp processing breakout group was reasonably certain that there is a medium probability of entry potential from coastal landfills to estuaries.

3.2.2.2.3. ***Other pathways.*** The other pathways breakout group found that the entry potential of viruses in ballast water, bait shrimp, and animal vectors is high. The group determined that while it is not likely that the freezing process used for bait shrimp will significantly reduce the virulence and infectivity of the virus, the effects of freezing may be virus specific. For shrimp feed, breakout group participants concluded that the probability of survival in transit depends on whether or not the feed meal is heat treated to temperatures sufficient to inactivate all viruses. It is thought that some of the viruses (e.g., TSV) may survive and maintain infectivity, even when heated to temperatures greater than 100 °C. While most of the fish meal produced in the United States is subjected to temperatures that appear to be sufficient to kill the viruses, breakout group members were unable to provide published data that would confirm this supposition. Moreover, several participants believed that other countries do not always heat-treat their meals, which would increase the potential for viable viruses to be present in the feed. The other pathways breakout group concluded that the transit survival probability is low for heat-treated feed and high for untreated feed. In contrast, the shrimp processing breakout group was very certain that feed was processed at temperatures sufficient to inactivate the viruses. Additional research will be necessary to resolve this issue.

3.2.2.3. *Colonization Potential*

Workshop participants agreed that the potential for viruses to colonize coastal areas is one of the most critical aspects of evaluating the potential for establishment. Workshop participants concluded that there is a high potential for viruses to be associated with many of the pathways identified in this report, but a low to high potential that these pathways could lead to introduction of viruses. The breakout groups were certain about association of viruses with these pathways and their entry potential through the pathway; however, they had a high degree of uncertainty about colonization potential.

3.2.2.3.1. ***Aquaculture.*** Many members of the aquaculture breakout group concluded that colonization potential was low (very uncertain). The rating of low was based on a lack of evidence that viruses had become established in wild shrimp populations in the United States as a result of aquaculture. Breakout group participants noted that colonization potential is likely virus specific and dependent on shrimp species and specific life stage. However, some breakout group members ranked colonization potential as medium, particularly for pond effluents, noting that these could provide continuous potential input of virus in coastal systems and as high if aquaculture expands further along the Gulf coast.

3.2.2.3.2. ***Shrimp processing.*** Colonization potential varied with pathway. Breakout group members considered colonization potential low for treated effluent, and they were very certain of this because they believed the disinfection processes would kill viruses. Colonization potential was also considered low for solid waste in landfills, but here there was reasonable uncertainty because of the absence of virus to shrimp dose-response data and the uncertainties associated with frequency and concentration of viruses associated with vectors at landfills. Shrimp and fish feeds were considered to have low colonization potential with high certainty because high temperatures used in processing feed were believed sufficient to kill pathogenic viruses. Colonization potential was judged to be medium for untreated effluent with medium uncertainty, because persistence, infectivity, and virulence of viruses in receiving waters will vary depending on numerous factors.

3.2.2.3.3. ***Other pathways.*** Colonization potential ranged from low to high depending on the pathway. For ballast water, colonization potential was considered low (moderately certain). On the basis of experience with other organisms, few organisms introduced into new environments survive to colonize. For penaeid shrimp and viruses, colonization depends on the point of discharge (e.g., nearshore vs. open ocean) and a number of other factors such as transmission and infectivity that are poorly understood outside of laboratory or aquaculture situations. For shrimp/fish food, colonization potential was thought to be medium (very uncertain). Introduction could occur via food used in aquaculture or chumming. The potential for viruses to colonize as a result of introduction from animal vectors was thought to be medium to high (relatively uncertain). Shrimp are likely to feed on bird feces that may contain viruses. Likelihood would increase in areas of high vector density (e.g., many seagulls where a shrimp die-off has occurred in an aquaculture pond). Finally, breakout group members thought that colonization potential from infected bait shrimp would be high (very uncertain) because bait shrimp are deposited in areas where native shrimp are known to occur.

In general, breakout groups believed that, for most subpathways, there is either a low or medium likelihood that, once introduced, viruses would be able to colonize native shrimp at a local level (i.e., within specific estuaries or embayments). The exceptions were high likelihoods of colonization noted for bait shrimp and, in the view of a few, aquaculture. In support of their conclusions, the breakout groups identified the following factors:

- Colonization potential is likely to be related to the magnitude of the source and the frequency of introductions. Therefore, large, frequent sources may have a greater likelihood of colonization than small, intermittent sources.

- Colonization potential is likely to be related to the medium in which the viruses are introduced. For example, viruses introduced within live or dead shrimp are thought to have a greater likelihood of colonization than are viruses introduced via water.

- There is no clear evidence to suggest that colonization has occurred in wild shrimp populations, despite a history of outbreaks in aquaculture operations, the presence of shrimp processing operations, discharges of ballast water, and the use of bait shrimp. (Although recent evidence suggests that WSSV-like viruses found in wild shrimp populations in South Carolina coastal waters may not differ from Asian isolates of the virus [Lo et al., in press], the significance of this observation is unclear.)

3.2.2.4. *Spread Potential*

The breakout groups viewed the potential spread of viruses beyond the initial locus of colonization as an area of great uncertainty. The aquaculture breakout group did not reach consensus on spread potential; estimates ranged from low (relatively uncertain) to high (very certain). Although viral diseases can spread rapidly between aquaculture ponds, participants recognized the difficulty in extrapolating from the spread of disease in aquaculture farms to that in wild populations. Factors such as population density and the time course of the disease may be important. For shrimp processing, spread potentials were judged to be low for both treated effluent (very certain, due to destruction of viruses by disinfection) and shrimp and fish feeds (very certain, because participants believed that high temperatures used in processing feed would kill pathogenic viruses). Spread potential for untreated effluent was considered medium (moderate uncertainty; persistence, infectivity, and virulence of viruses in receiving waters are sources of uncertainty and will vary depending on numerous factors). The spread potential for solid waste in landfills was considered high (reasonably certain). The other pathways breakout group believed that colonization potential for the four main pathways it considered was medium (very uncertain). During plenary discussion of the reports from the individual breakout groups, workshop participants generally believed that there is a medium probability that viruses could spread beyond the initial locations of colonization.

Breakout groups identified a number of factors significant to evaluating the potential spread of introduced viruses, such as the degree of interaction that would occur among individual wild shrimp and the spatial scale over which shrimp might "mix." Stocks of *P. setiferus* in the southeast Atlantic are thought to be fairly genetically homogeneous, as are the northern and southern populations in the Gulf of Mexico. Workshop participants believed that this suggests the potential for substantial interaction over broad geographic regions, which would promote the spread of viral infection. However, genetic homogeneity may not be the case for other penaeid species. The potential for spread also depends in large part on the time course of the disease, as well as the density of shrimp in wild populations. Breakout group members determined that low shrimp densities are likely to hinder disease spread, whereas high densities are likely to promote transmission. Spread potential is also host dependent and virus specific. It was noted that TSV and IHHNV have low spread potential, and the spread potential of YHV and WSSV is currently unknown. A WSSV-like virus has been found in a variety of crustaceans in southeastern Atlantic waters, but it is unknown at this time if it is the same as the Asian strain of WSSV. (Recent evidence suggests, however, that WSSV-like viruses found in wild shrimp populations in South Carolina coastal waters may not differ from Asian isolates of the virus [Lo et al., in press]). This evidence suggests a potential for colonization and spread, but it is unclear whether the WSSV-like viruses are indigenous, or if nonindigenous, when they may have been introduced. Finally, as noted in the JSA report, the presence of other stressors (e.g., low dissolved oxygen and extreme salinity) is also likely to influence the potential for spread of the disease.

3.2.3. Consequences of Establishment

In continuing to assess the risks to wild populations of shrimp viruses, the breakout groups evaluated the potential ecological effects associated with the establishment of pathogenic shrimp viruses. The breakout groups approached this step of the qualitative risk assessment process by considering the available information on the direct effects of viruses on shrimp. Breakout groups also examined possibly analogous situations based on experience with other diseases and invertebrates. Breakout groups discussed possible effects on ecological structure and function but, due to the limited time available, gave primary attention to direct effects on wild shrimp populations. In the absence of documented information or firsthand knowledge, experts relied primarily on professional judgment to evaluate the consequences of establishment. The breakout groups concluded that there is a high degree of uncertainty in assessing the consequences of establishment.

3.2.3.1. *Direct Consequences to Shrimp Populations*

In considering the possible consequences of shrimp viruses to shrimp populations at the local level and at the scale of the entire populations or stock, breakout groups evaluated three types of effects:

- Mortality of the infected animal
- Reduction in reproductive rates
- Alteration of the genetic structure of the population

3.2.3.1.1. ***Mortality effects.*** Breakout group experts concluded that the direct consequences of the establishment range from low to medium and that effects on the mortality of shrimp are more likely to occur at the local level than at the scale of the entire population or stock. The breakout groups determined that the probability of colonization at a local level is greater than the probability that viruses would spread beyond the local level to a regional population. It is thought that WSSV and YHV are more likely than IHHNV or TSV to cause acute mortality but that IHHNV and TSV are more likely to become endemic following introduction.

3.2.3.1.2. ***Reproductive effects.*** Breakout group experts focused primarily on factors that would affect reproductive output or recruitment. Experts were aware of no information describing adverse viral effects on the reproductive potential of infected individuals (indicating a potentially important data gap). One participant noted that reproductive output of infected *P. vannamei* brood stock appears to be unaffected by viral infection. However, in contrast to the previous statement, individual growth impairment in offspring of *P. vannamei* infected with IHHNV has been documented (Fulks and Main, 1992). Assuming that fecundity of female *Penaeus* is an increasing function of size (a phenomenon common in other invertebrate species), workshop participants considered that stunted growth of offspring could result in reduced reproductive output of the second generation. Individual growth impacts could therefore cause population-level effects, although an analysis of any changes in reproduction on shrimp population dynamics would be required to support this assertion. Workshop participants noted that epidemiologic models show that in “r-selected” species, effects on reproduction can have greater effects on population size than mortality effects. (Penaeid shrimp can be characterized as “r-selected” organisms because they display an annual life history pattern with high reproductive output and high mortality during early life stages.)

3.2.3.1.3. *Effects on genetic structure and fitness.* Breakout group participants discussed the potential effects of virus colonization on the genetic structure and fitness of wild shrimp populations. One breakout group thought that rapid reductions in population abundance resulting from viral disease could have unknown but potentially important effects on genetic structure by limiting genetic variability (the "founder effect"). One participant cited evidence from Thailand indicating that shrimp populations in the south of Thailand are much less genetically diverse than those from the northern part of the country. It has been hypothesized that this is due to the release of shrimp from aquaculture into the wild. One breakout group discussed the importance of understanding whether genetic resistance to viruses differs among populations. Further knowledge of genetic variability among Gulf Coast shrimp is necessary to make accurate predictions about which area has the highest potential for an epizootic.

3.2.3.1.4. *Other information.* Other information or lines of evidence that affected the experts' professional judgments about the potential consequences of establishment are summarized below:

- Penaeid shrimp can be characterized as "r-selected" organisms because they display an annual life history pattern with high reproductive output and high mortality during early life stages. Thus, penaeid shrimp populations that suffer population reductions in one year can exhibit rapid recovery, and this may reduce the long-term consequences of short-term impacts. In reviewing available information, the breakout group concluded that mass mortalities of adult shrimp may have relatively short-term impacts on standing shrimp stocks. For example, some natural stressors on shrimp (e.g., cold temperatures or freshwater flooding) are known to cause short-term reductions in populations at the local level. Because of high fecundity and migratory behavior, *P. setiferus* is capable of rebounding from a very low population size in one year to a large number in the next, if environmental conditions are favorable. This has been observed off the South Carolina coast several times in the past 50 years (Linder and Anderson, 1956; McKenzie, 1981). In another case, an increase in reproductive output of the Honduran population of *P. vannamei* was reported during a 1994 TSV outbreak. This provides anecdotal support for the concept that demographic compensatory responses may occur in disease-depleted populations, although it was noted that the population changes could have been caused by other factors (Laramore, 1997).

- Along with anecdotal information about the possible long-term effects of viral infections in Latin American and Asian shrimp populations, observations by some workshop participants indicated that direct mortality effects could be relatively transitory. Also, based on the observation that resistance to IHHNV appears to have increased in all populations tested since the identification of this virus in Hawaiian stocks, it was suggested that initial outbreaks could lead to enhanced resistance to future viral infection.

It should be noted, however, that some workshop participants were concerned that the ability of viral pathogens to persist at low levels in a population could result in long-term adverse population effects. For example, participants noted the purported virus-induced declines in the population abundances of *P. stylirostris* in the Gulf of California began in 1987 and lasted 6 to 7 years, with stocks now reported to have returned to preoutbreak levels. (The role of IHHNV as the cause of the initial population decline has been the subject of much debate, however.)

- Based on observations from aquaculture situations, it appears that local colonization of shrimp viruses could result in local mortalities of shrimp. For example, TSV and others viruses are known to cause mass mortality on shrimp farms. Experiments with these viruses have documented mortality rates of up to 100%. One participant noted that in South Carolina, survival on commercial farms affected by TSV dropped from 63% in 1995 (the year prior to the TSV outbreak) to 19% in 1996 (the year of the TSV outbreak).

- Lines of evidence from other crustacean species indicate an association between an introduced biological agent and subsequent environmental impacts. For example, a crayfish species introduced from California to Europe may likely have served as a carrier to spread the freshwater crayfish plague throughout Scandinavia (Unestam and Weiss, 1970). Unlike short-term natural stressors (e.g., changes in temperature or salinity), an introduced disease organism (biological stressor) is likely to persist in the population.

- No empirical data exist to indicate that historical releases of shrimp virus to the Gulf of Mexico or to southeast Atlantic coastal waters have resulted in population-level impacts. However, no well-designed studies have been conducted to examine the epidemiologic conditions within these waters.

3.2.3.2. *Effects on Ecological Structure and Function*

Workshop participants observed that the introduction of nonindigenous shrimp viruses could affect ecological conditions apart from any direct effects on shrimp. Because these indirect effects were not a focus of this workshop, experts made only a limited attempt to characterize these consequences. Despite these limitations, some of the discussion related to this topic may be helpful to risk managers and is included in this report.

The aquaculture breakout group discussed instances in which other invertebrate species have experienced severe disease consequences. Participants viewed these examples as relevant to the effects of nonindigenous pathogenic viruses on shrimp:

- The near decimation of oysters (*Crassostrea virginica*) by the protozoan pathogens *Haplosporidium nelsoni* and *Perkinsus marinus*, called MSX and dermo disease, respectively (Haskin and Andrews, 1988; Andrews, 1996; Burreson and Ragone-Calvo, 1996), has resulted in significant changes in the oyster reef habitat throughout Chesapeake Bay and dramatically reduced the rate at which bay water was filtered by feeding bivalves (Kennedy, 1996).

- Insect/virus associations were described in which high abundances of the host species promote rapid outbreaks of viral disease, followed by dramatic declines in the host, near disappearance of the virus, and reestablishment of the host.

- The introduction into Scandinavia of North American crayfish that were carriers of the freshwater crayfish plague *Aphanomyces astaci* (Unestam and Weiss, 1970) had significant consequences.

Workshop participants believed that, in the absence of data on nonindigenous shrimp viruses in the wild, these and similar examples could serve as models for extrapolating potential consequences of viral establishment for shrimp populations. These examples may also serve as models for how ecological systems might be affected by viral outbreaks in shrimp. Either application would require careful analysis to identify similarities and differences relative to the shrimp virus situation.

The other pathways breakout group discussed the potential for viruses to affect estuarine ecology by infecting other species of shrimp, such as grass shrimp. Grass shrimp (*Paleomonetes sp.*) are an important part of the estuarine food web. Many species of fish (and penaeid shrimp) rely on this species as an important prey item. Data from Thailand suggest that grass shrimp may be carriers of one or more of these viruses, but data on infectivity rates and effects for Thai grass shrimp are lacking. On the other hand, it was noted that observations in South Carolina confirmed the presence of large populations of apparently healthy *Paleomonetes* in tidal areas near TSV-infected shrimp farms.

3.2.4. Risk Characterization

Using the ANSTF approach (ANSTF, 1996; Appendix G), workshop participants characterized the risk of viral introductions to wild penaeid shrimp populations by combining the probability of establishment of the virus with that of the presumed ecological consequences (see Section 3.1). Workshop participants assessed risks to local populations, which the experts generally defined as the population within a single estuary; they also considered the long-term effects on the entire population of native shrimp in the Gulf of Mexico and southeastern Atlantic coastal waters.

The risks estimated by the individual breakout groups are summarized in Tables 2 through 5. The discussion in this section is based on those risk estimates but emphasizes overall conclusions drawn during plenary workshop discussions among all breakout group participants.

3.2.4.1. *Risk to Local Populations*

Workshop participants concluded that the probability of establishment of shrimp viruses in a local estuary ranges from low to medium. The probability of establishment depends primarily on the colonization potential of the particular viruses. However, the probability of establishment could become much greater if virus is introduced repeatedly to the estuary over a long period. Workshop participants generally believed that the impact of such an establishment on the local shrimp population might involve high initial kill rates followed by rapid recovery due to reintroduction of shrimp from other locations. Therefore, workshop participants characterized the overall long-term risk of nonindigenous pathogenic virus introductions to the shrimp populations in a local estuary as generally low to medium. (The possibility of longer-term effects is suggested and discussed in Section 3.2.3.1).

Although workshop participants had very little time to consider the risks posed by nonindigenous shrimp viruses to other components of the estuarine ecosystem, many believed the level of risk to be medium, although uncertainty surrounding this risk estimate is very high. Of particular concern to participants was co-infection of important food web species, such as grass shrimp and crayfish. Because both penaeid shrimp and grass shrimp are important food sources for many other estuarine organisms, participants noted that the loss of this food base could have significant effects on other species. Following an initial viral kill of shrimp, fish or wildlife populations that depend on shrimp and other crustaceans as prey sources may take longer to recover than shrimp populations.

Participants raised concerns about the lack of information on the transmissibility of disease from one estuary to another through migration of diseased or infected shrimp. Participants thought that survivors of a local epizootic could move out to sea to reproduce, possibly infect other shrimp and offspring, and then move into adjacent or nearby estuaries. Such an event would expand what appears to be a localized risk into large-scale risk; however, each breakout group that evaluated the potential for spread by natural processes rated the probability of this occurrence as low. Therefore, the risk of a local infection having large-scale consequences is characterized as medium.

3.2.4.2. ***Large-Scale Risk***

Workshop participants characterized the risk from viral introductions to the entire population of native shrimp along the southeastern Atlantic coast and within the Gulf of Mexico using the same analysis of the establishment pathways combined with that of the potential consequences of establishment on a large geographic scale. Workshop participants concluded that the consequences of virus introduction to the population as a whole would be relatively insignificant, and they characterized the risk as low.

Some participants expressed concern that the genetic structure of the population might be altered, and if viral resistance were linked with certain other important genes, overall fitness of the shrimp could be lowered. One participant noted that alterations to the genetic structure of the population could make the shrimp more susceptible to future infections and to simultaneous environmental stressors, such as weather changes or reduced estuarine salinity, thereby potentially increasing the risk potential. Furthermore, some participants stressed that uncertainty about the long-term ecological consequences of viral introduction will remain high until the effects of virus infection on reproduction can be determined.

3.2.4.3. ***Summary***

Overall conclusions by workshop participants concerning the risks posed by nonindigenous pathogenic shrimp viruses may be summarized as follows:

- Based on information currently available, most workshop participants believed that the risk to native shrimp from introduction of nonindigenous viruses is low to medium, although uncertainty is high.

- Most participants agreed that local effects should be given a higher risk ranking than large-scale effects because local effects are more likely to occur.

- Participants suggested that the large amount of uncertainty associated with this risk characterization could be reduced through appropriate laboratory and field studies. The lack of evidence of conclusive viral impacts on worldwide shrimp populations does not derive from published systematic studies but rather is anecdotal. Furthermore, by analogy, other marine invertebrates have experienced severe local impacts from exposure to pathogens (as has been noted in oyster populations in Chesapeake Bay). Also, viruses that have become established in terrestrial insect populations can cause cyclic epizootics and population crashes. Therefore, participants concluded that there is an urgent need to continue efforts to gather available data on shrimp virus effects and to conduct a systematic research effort that could be used to reduce the uncertainty of any subsequent risk assessments.

3.3. RISK MANAGEMENT RELEVANCE

Although this report does not recommend risk management actions, it contains information that may help risk managers with their decisions by:

- Providing insight into the pathways by which shrimp viruses could potentially enter and become established in the marine environment
- Identifying potential consequences to wild shrimp populations at local and stock levels
- Suggesting specific actions and studies that can reduce the uncertainties associated with evaluating the potential risks of shrimp viruses on wild shrimp populations

The ability to make quantitative estimates of the risks of viruses to wild populations of penaeid shrimp is constrained by the amount and type of information that is currently available. The majority of workshop participants believed that it is unlikely that the information required to complete a quantitative risk assessment will be available within the foreseeable future. At present, qualitative evaluations can be made.

The ability of workshop participants to address broader ecological risks in a comprehensive manner was limited by available information, but participants agreed that this important issue merits further consideration. Furthermore, while the topic of risks that nonindigenous pathogenic shrimp viruses pose to shrimp aquaculture operations was not part of the scope of the workshop, workshop participants agreed that these risks should be given special attention as part of another technical or management workshop.

4. ACTIONS FOR REDUCING UNCERTAINTY

The qualitative risk assessment conducted during the workshop revealed several critical sources of uncertainty. Further improvement in the ability to estimate risks to wild populations of shrimp will require reducing uncertainty in these key areas.

Workshop participants discussed the relative importance of actions for reducing uncertainty. Some participants stressed that, to reduce uncertainty, risk management actions need to occur in parallel with research, monitoring, and other actions. Most workshop participants generally believed that particular emphasis should be given to the following actions for reducing uncertainty:

- Improved diagnostic methods
- Surveys of wild shrimp populations for the presence of nonindigenous viruses and for genetic composition
- Experiments to reduce uncertainties surrounding virus transmission and virulence
- Field epidemiological studies

4.1. DIAGNOSTIC METHODS

Workshop participants determined that improvements to existing diagnostic methods and development of new diagnostic tools are very high priorities. Several participants noted that without adequate diagnostic methods, other risk assessment elements cannot be well studied or adequately evaluated. Other participants noted that many valuable diagnostic tools currently exist. Several key needs were identified during the workshop:

- There is a significant need to develop new diagnostic procedures. Some molecular probe applications and bioassay tests are available, although several workshop participants noted that the sensitivity of existing bioassay tests needs to be improved. One participant also cited the need to develop cell culture tests for crustacea, noting that new technologies are available to assist in developing cell cultures, but money and lack of equipment have been major obstacles.

- Tests for infectivity are needed to establish the threshold number of viruses that would be required for colonization potential. At least two tests should be employed, such as a PCR and ELISA or a PCR and a bioassay.

- Current diagnostic applications are focused on detecting viruses in the animal itself. Although some preliminary efforts have been made to detect viruses in environmental media (e.g., to identify the presence of WSSV using water concentration techniques and PCR), techniques to detect viruses in effluent streams, sediment, and other environmental media need to be improved.

- There appears to be considerable variability among laboratories in the procedures for using available diagnostic tools. Procedures for using diagnostic tools should be standardized so that both the credibility and limitations of diagnostic tools can be established.

4.2. SURVEYS OF WILD SHRIMP POPULATIONS

Participants identified the need to survey native shrimp populations to develop baseline information on viruses in wild stocks. It was noted that some monitoring activity has been conducted in the coastal waters of South Carolina and Texas. Participants generally believed that it was important to proceed with field surveys despite the current limitations of diagnostic methods. Participants suggested that because of these limitations, current survey efforts should include the archiving of samples to be evaluated pending development of improved diagnostics.

Workshop participants noted that monitoring surveys should include genetic characterization of wild populations. To date, only limited studies have been conducted. (In one study that is under way, molecular techniques are being used to determine the degree of genetic variability between populations of *P. setiferus* in the Gulf of Mexico and the U.S. southeastern Atlantic coastal region.) Participants suggested that surveys should be focused both in areas that may have experienced the release of nonindigenous viruses and areas where it is unlikely that prior release has occurred.

4.3. EPIDEMIOLOGY OF SHRIMP VIRUS TRANSMISSION

Workshop participants identified a need for well-designed experiments to improve understanding of the pathogenicity of viruses in native shrimp. In particular, studies are needed on virulence, distribution in various shrimp tissues, and rates of transmission, susceptibility, and recovery. Some suggested that laboratory experiments would be hindered by inadequacies in current techniques to identify pathogens and by the absence of diagnostic methods specific to identifying viruses in various environmental media. Given existing techniques for quantifying the amount of virus present, participants noted that currently it is most feasible to conduct qualitative transmission studies in which the amount of virus is estimated on a relative basis.

In other discussions, participants identified the need to understand not only mortality effects but also the consequences of infection on shrimp reproduction and growth. It is recognized that there are significant differences in viral pathogenesis among the four different viruses and the relative ability of the viruses to affect mortality, growth, and reproduction.

Participants also identified the need to develop a better understanding of the transmission of viruses from one species to another (i.e., between penaeid species and between penaeid and nonpenaeid species).

One participant stated that the most important reason to improve understanding of the epidemiology of shrimp viruses is to help identify mitigation measures (e.g., for aquaculture as a pathway).

4.4. FIELD EPIDEMIOLOGIC STUDIES

In addition to laboratory-based experiments, most participants believed that a parallel effort involving field epidemiology could yield information helpful for understanding the prevalence and potential effects of viruses in wild shrimp populations. Field epidemiologic studies may not provide the same level of understanding of detailed mechanisms as would laboratory experiments.

Participants suggested that field epidemiologic studies could make use of existing information from Latin America and Southeast Asia. Information would be sought on:

- The extent to which native shrimp populations in these areas may have been exposed to viruses
- The presence of viruses within these populations
- The observed effects (or lack thereof) of viruses on shrimp abundance and recruitment
- Possible ecological effects

Others suggested that the known locations of shrimp virus prevalence around the world should be documented and mapped so that potential sources can be identified.

4.5. LOWER PRIORITY RISK-RELEVANT RESEARCH AREAS

Workshop participants identified other areas, in addition to the four priority areas listed previously, where additional research is needed to improve the ability to estimate risks to wild shrimp populations.

4.5.1. Viral Persistence

Some participants noted the need to develop better techniques and to conduct experiments to evaluate the persistence of viruses in effluent streams, sediment, and other environmental media. It was noted that experiments should couple viral persistence with viral infectivity. For example, participants noted that IHHNV can be detected in sediments for 24 days; however, the duration of infectivity is unknown.

4.5.2. Compensatory Mechanisms

Participants believed that it is important to develop a better understanding of the compensatory mechanisms of native shrimp species in response to viral disease outbreaks. Research is needed to:

- Understand genetics and disease resistance (i.e., the need to improve understanding of the relationship between population genetics and the identification of disease-resistant phenotypes and how particular phenotypes develop resistance to a particular virus).

- Determine whether shrimp populations compensate for increased mortality with increased reproduction.

- Compile information on the shrimp immune-like response to viral infection. It was noted that coupling our understanding of target-organ sensitivity with information about resistance will improve the ability to predict which shrimp are likely to become carriers.

4.5.3. Monitoring of Imported Shrimp

Participants identified the need to monitor virus levels in imported shrimp using tests such as PCR and bioassay. Some experts suggested that, in terms of risk reduction, monitoring imported shrimp should be a higher priority than monitoring wild shrimp populations because of the high volume of imported shrimp.

4.5.4. Development of Suitable Population Models

Suitable population models are needed to evaluate the consequences of various virus-induced mortality or reproductive impairment scenarios. Because of the commercial importance of shrimp, workshop participants believed that it is highly likely that population models exist for these species. Additionally, a large body of catch statistics could be subject to time series analysis in concert with known periods of virus outbreaks or other environmental stressors, such as storm events. These types of data may be available for foreign fisheries as well. By using population models, constants for infection and transmission rates, and transport and fate, a modeling framework could be created to examine specific hypotheses. Sensitivity analyses could then be performed to determine which parameters are most important and contribute the most uncertainty. Research could then be directed to reduce uncertainty.

4.5.5. Other Risk-Related Research Needs

Other risk-related research needs identified by workshop participants include:

- Procedures for disinfection and eradication of large-scale outbreaks in aquaculture settings
- Genetic and biochemical characterizations of the viruses
- Research to improve understanding of factors that exacerbate expression of viral disease under conditions of high densities and high nutrients found in aquaculture settings
- Targeted surveys of nonpenaeid species (e.g., grass shrimp, crayfish, and micro-crustacea) to determine if they are susceptible to, or carriers of, nonindigenous viruses

5. SUMMARY

This section provides a brief summary of the results of the workshop. Topics include the qualitative risk assessment process; the need for a future, more comprehensive risk assessment; risk-relevant research needs; and areas of additional concern.

5.1. QUALITATIVE RISK ASSESSMENT PROCESS

Workshop participants conducted a qualitative assessment of risks by considering the:

- Likelihood of viruses being present in the pathway
- Ability of the viruses to survive transit in the pathway
- Colonization potential of the viruses (in native shrimp)
- Spread potential of the virus within native shrimp populations
- Consequences of establishment

In general, workshop participants believed that viruses could be in pathways leading to coastal environments and that they could survive in these pathways. Participants concluded that there is some potential for viruses to colonize native shrimp in a localized area, such as an estuary or an embayment, near the point of entry into the marine system. Participants had widely divergent views on the potential for viruses to spread beyond the initial local area of colonization, and this divergence reflected the large uncertainty associated with this aspect of exposure. Participants considered the potential for localized colonization and subsequent spread to be a critical aspect of evaluating the potential establishment of viruses in native shrimp.

Workshop participants considered the consequences of virus establishment at a local level (e.g., within an individual estuary) as well as within the offshore stocks. Participants discussed the impact of such an establishment on the local shrimp population. Initial kill rates might be high, but the population would be likely to recover rapidly due to reintroduction of shrimp from other locales. Workshop participants characterized the risk from viral introductions to the entire population of native shrimp along the southeastern Atlantic coast and within the Gulf of Mexico as low. Concern was expressed that certain effects (e.g., effects on genetic structure of shrimp and on the ecological system) may be difficult to assess.

5.2. COMPREHENSIVE RISK ASSESSMENT NEEDS

Most workshop participants concluded that, given the current knowledge base, it is infeasible to conduct a more comprehensive, quantitative estimate of risk. Most participants believed that, at present, qualitative evaluations can be made, but these are accompanied by large uncertainties. Participants agreed that there is a need to continue efforts to gather available data on shrimp virus effects and to conduct a systematic research effort that could be used to reduce the uncertainty in any subsequent risk assessments.

5.3. RESEARCH NEEDS

Workshop participants identified a number of areas in which further research and information would improve the assessment of risks and the evaluation of current conditions, with particular emphasis on the following areas:

- **The improvement of existing and the development of new diagnostic methods for viruses in shrimp and environmental media.** These methods are essential for all research studies and monitoring programs and for determining if viruses are present in imported shrimp, cultures used for aquaculture, and other possible pathways.

- **Surveys of wild shrimp populations.** Baseline information on the presence of viruses in native shrimp populations would provide insight into the extent to which populations already carry viruses. Baseline information would also be useful for supporting epidemiologic studies. Baseline studies could proceed even though there are limitations with current diagnostic methods. Well-designed studies would be enhanced by including an examination of the genetic structure of the populations.

- **Epidemiology of shrimp virus transmission.** Workshop participants identified a need for well-designed experiments to improve understanding of the pathogenicity of viruses in native shrimp.

- **Field epidemiologic studies.** In addition to laboratory-based experiments, participants believed that a parallel effort involving field epidemiology could yield information helpful for understanding the prevalence and potential effects of viruses in wild shrimp populations.

5.4. ADDITIONAL AREAS OF CONCERN

Workshop participants identified the following areas of concern, in which additional efforts should be focused:

- **Management implications of shrimp viruses.** It was recommended that a risk management workshop be held, focusing on impacts to natural resources and on possible impacts on shrimp importation, processing, and aquaculture operations.

- **Risks of shrimp viruses to aquaculture operations.** Workshop participants also recommended that a separate workshop be held on this topic.

- **Risks of shrimp viruses to nonpenaeid species.** Because this workshop was limited to evaluating the direct effects of viruses on wild shrimp populations, participants recommended that additional effort be directed toward evaluating nonpenaeid shrimp species (e.g., grass shrimp) and other species (e.g., crabs, amphipods, and copepods) that could be impacted by viruses.

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APPENDIX A

BREAKOUT GROUP REPORTS

APPENDIX A. BREAKOUT GROUP REPORTS

Workshop participants were organized into three groups, each of which was charged with evaluating the risks associated with one of the following categories of viral pathways:

- Aquaculture
- Shrimp processing
- Other potential pathways

Dr. Wayne Munns (EPA Office of Research and Development) led the aquaculture group, Dr. John Gentile (University of Miami) led the shrimp processing group, and Dr. Anne Fairbrother (Ecological Planning and Toxicology, Inc.) led the "Other Pathways" group. Prior to the workshop, participants were given their breakout group assignment (Appendix B) and provided premeeting materials for their consideration in preparing for the workshop (Appendix C). At the discretion of each breakout group chair, observers were provided an opportunity to participate in discussions during breakout group sessions.

The breakout groups applied an adaptation of the risk assessment procedure described in the Aquatic Nuisance Species Task Force (ANSTF) report (RAM, 1996; Appendix G) to evaluate the ecological risks associated with each identified viral pathway (see also Section 2.1). Each breakout group evaluated and ranked elements of both the potential for establishment of the viruses via the identified pathways and the potential ecological consequences of establishment, should it occur. Breakout groups also identified the level of uncertainty (ranging from very uncertain to very certain) associated with these rankings.

After the workshop, Dr. Munns prepared the report of the Aquaculture Breakout Group (Appendix A-1), Dr. Gentile prepared the report of the Shrimp Processing Breakout Group (Appendix A-2), and Dr. Fairbrother prepared the report of the "Other Pathways" Breakout Group (Appendix A-3). Workshop participants had a chance to review and comment on the breakout group reports prior to preparation of the final document.

A-1. Report of the Aquaculture Breakout Group

A.1.1 INTRODUCTION

This breakout group was charged with assessing the risk associated with introduction of nonindigenous virus to wild shrimp populations from the shrimp aquaculture pathway (see Figure A-1).

Prior to implementing the ANSTF process, the Aquaculture Breakout Group addressed two questions. First:

1. Should the evaluation consider the four primary viruses (IHHNV, TSV, WSSV, and YHV) separately or as a group?

The breakout group recognized that consideration of differences among the viruses and in their relationships with host penaeids could lead to different ratings of the elements comprising probability of establishment; however, given the time constraints for completing the risk assessment, the breakout group decided that the viruses would be considered as a group whenever possible, but unique differences would be identified that might contribute to distinctly different conclusions about elements of the probability of establishment.

The second question addressed by the group was:

2. Should the evaluation consider risks of viruses directly to aquaculture operations in addition to the two assessment endpoints identified in the JSA report?

In its initial deliberations, the breakout group noted that aquaculture operations have already experienced outbreaks of viral infection, some of which have been catastrophic. This suggests that, because of the obvious risks to aquaculture, a further assessment to estimate these risks is not necessary at the present time. The breakout group decided instead to recommend to risk managers that action is needed to minimize risks to aquaculture from future outbreaks. Effective mitigation of this risk is likely to require evaluation of viral pathways to aquaculture operations; therefore, some future pathway analysis may be necessary. For this assessment, the breakout group decided to consider sources and pathways leading to aquaculture only if they provided information relevant to aquaculture as a source of viruses to wild populations of shrimp.

A summary of risk ratings discussed by the aquaculture breakout group is provided in Table A-1.

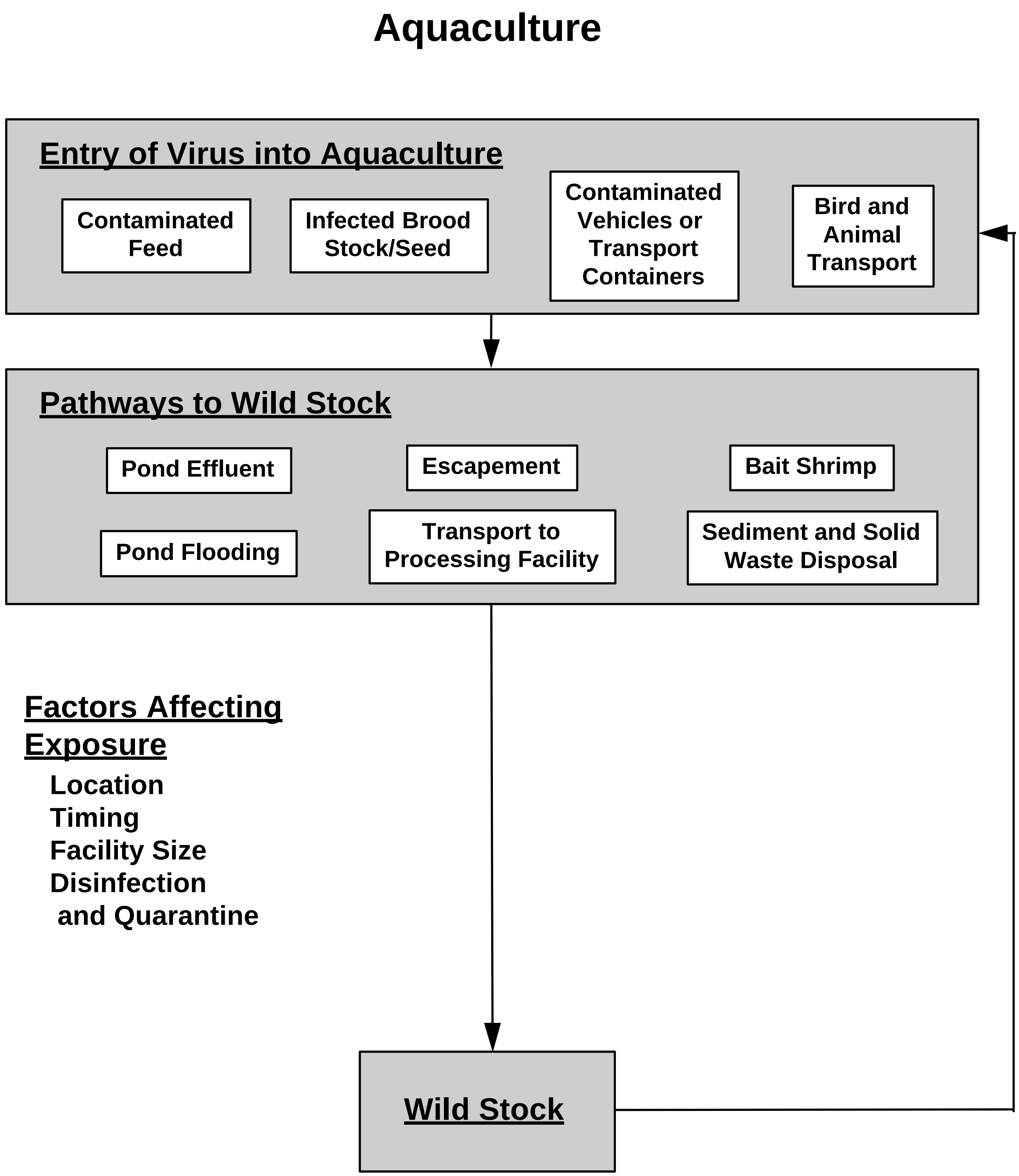

Figure A-1. Conceptual model: Virus sources and pathways for aquaculture (JSA, 1997)

Table A-1. Summary of Aquaculture Breakout Group risk rankings

Refer to supporting discussion in the text to properly evaluate information presented in this table. The risk assessment process is described in Section 2.1 and Appendix G.

Probability of Establishment	Pathways to the Environment: Escapement	Pond Flooding	Pond Effluent	Transport to Processing Facility	Sediment and Solid Waste Disposal
Association with Pathway	High/very certain	High/very certain	High/very certain	High/very certain	High/very certain
Entry Potential	High/very certain or low/reasonably certain[1]	Low/ very certain	Medium/very certain	Low/ reasonably certain	Low/ reasonably certain
Colonization Potential	Low (or medium to high)[2]/very certain	Low (or medium to high)[2]/very certain	Low (or medium to high)[2]/very certain	Low (or medium to high)[2]/very certain	Low (or medium to high)[2]/very certain
Spread Potential	Low/relatively uncertain to high/very uncertain[3]	Low/relatively uncertain to high/very uncertain[3]	Low/relatively uncertain to high/very uncertain[3]	Low/relatively uncertain to high/very uncertain[3]	Low/relatively uncertain to high/very uncertain[3]
Overall Probability of Establishment	Low to high	Low	Low to medium	Low	Low
Consequences of Establishment	Low to medium/very uncertain	Low to medium/very uncertain	Low to medium/very uncertain	Low to medium/very uncertain	Low to medium/very uncertain
Overall Risk Estimate	Low to high	Low to medium	Low to medium	Low to medium	Low to medium

[1] High if pond is infected and shrimp escape from pond; low otherwise.

[2] Some breakout group members believed that the potential was medium and would be high if the aquaculture industry expands significantly along the Gulf Coast.

[3] The breakout group could not reach consensus; opinions on entry potential ranged from low to high.

A.1.2 PROBABILITY OF ESTABLISHMENT OF VIRUSES IN AQUACULTURE

A.1.2.1 Probability of Nonindigenous Viruses Being in the Aquaculture Pathway

The occurrence of nonindigenous viruses in U.S. aquaculture operations is well documented. The breakout group concluded that the probability of nonindigenous viruses being in the aquaculture pathway is **High (Very Certain).** As summarized in the JSA report, TSV has been identified in disease outbreaks in Hawaii, Texas, and South Carolina (Lightner, 1996a, 1996b). IHHNV was first identified in Hawaii (Lightner et al., 1983a, 1983b) and was subsequently observed in farms in South Carolina, Texas, and Florida (Fulks & Main, 1992). WSSV and YHV also have been documented at a shrimp farm in Texas (Lightner, 1996a, 1996b), and a WSSV-like particle has been identified in South Carolina (P. Sandifer, personal communication). Breakout group members noted that the origins of these viruses are not always traceable to their ultimate sources, but it was suggested that their introduction to the United States may have resulted from importation of infected shrimp from other regions of the world (e.g., Latin America and Asia). The breakout group questioned the frequency of virus occurrence in U.S. aquaculture operations due to the lack of well-established monitoring programs and detection protocols; the group concluded, however, that, given the time course of disease progression and the nature of current shrimp farming practices (e.g., high shrimp densities), it is very certain when viruses are present.

A.1.2.2 Probability of Nonindigenous Viruses Surviving in Transit in the Aquaculture Pathway

To determine the probability of nonindigenous viruses surviving in transit, the breakout group considered the six subpathways from aquaculture to wild shrimp stocks, as shown in Figure A-1. The group initially attempted to rate survival in transit for each subpathway in an effort to provide complete information for management consideration; however, there was insufficient time for this task and the group determined that the probability of surviving in transit is primarily a function of the most likely subpathway. Given the lack of information and high uncertainty for subpathways such as pond effluent and sediments, the breakout group tabled discussions of these and other pathways and focused much of their discussion on one remaining subpathway (escapement), which includes both accidental and intentional releases, as well as “escape” via transport of shrimp tissue by the predatory activities of other animals. (However, as discussed in the following, opinions diverged on this topic. Some breakout group members believed that the sediment and effluent pathways, which the group tabled because of a lack of crucial data, may also be important.)

A.1.2.2.1 Escapement Subpathway

Information relevant to this rating includes documented cases of shrimp escapement in South Carolina (South Carolina Department of Natural Resources, C. Browdy, personal communication) and capture of cultured species in Texas waters by shrimp trawlers (R. Goldburg, personal communication). The frequency of escapement is said to be low and infrequent because of engineering controls, such as the use of screens in effluent streams (F. Jaenike, personal communication). However, the breakout group recognized that the release of viruses to the environment via this subpathway is dependent on the life stage of the infected shrimp (e.g., larval stages may be more likely to bypass engineering controls). Professional judgment suggests that all life stages are capable of escape under favorable conditions.

The breakout group agreed that viruses would survive shrimp escapement. The group acknowledged that the probability of release of viruses to the environment is a function of the probability that a pond is infected and the probability of shrimp escaping from that pond. The breakout group concluded that the probability of surviving in transit would be **High (Very Certain)** if these two conditions were met but would be **Low (Reasonably Certain)** if they were not met.

A.1.2.2.2 Pond Flooding Subpathway

The breakout group concluded that the probability that nonindigenous viruses could escape aquaculture operations via pond flooding was **Low (Very Certain**), based on the judgment that ponds are unlikely to flood to overflowing. For example, ponds did not overflow during recent hurricanes in South Carolina, although the intensity of a storm event, its point of impact, and the specific location of aquaculture ponds would all influence the likelihood of flooding and the potential for escapement.

A.1.2.2.3. Pond Effluent Subpathway

Due to the lack of data and consensus among breakout group members, the breakout group did not complete an evaluation of this pathway, although a rating of **Medium (Very Uncertain)** was assigned. The primary uncertainties are the presence, viability, and infectivity of viruses in effluent waters. It was noted that TSV has been documented in water but not necessarily in effluent waters. There is suggestive evidence about this potential pathway. A workshop observer (R. Laramore) communicated results of an experiment that suggest that caged shrimp exposed in infected ponds developed disease. (Shrimp developed disease when exposed within 1 to 2 days to experimentally inoculated water, but they did not develop disease when exposed within 3 to 5 days of the water's inoculation [R. Laramore]). In 1995, HSF, Ltd., and the Arroyo Aquaculture Association conducted several trials in which cages were floated within a shrimp growout pond that had experienced a TSV epidemic and with pond water in tanks. The cages were suspended above the pond bottom and stocked with juvenile *P. vannamei.* No TSV was detected in shrimp exposed for 30 days under these conditions (F. Jaenike, personal communication). These results suggest that TSV may be transmitted during the acute but not the chronic stages of the disease. An unsubstantiated statement was made that viruses sorb quickly to particulate matter and, by so doing, may reduce their potential for future infection. The breakout group concluded that experiments critical to addressing this subpathway have not been conducted.

Some members of the breakout group offered a dissenting opinion about the potential for virus transmission in effluent waters. They believed that the group had not adequately evaluated this pathway. It was noted that data from J. Lotz suggest that IHHNV and TSV can survive in an infective state for a minimum of 28 days. D. Lightner suggested that IHHNV can survive in sediments for up to 24 days; however, he had not evaluated the virus's infectivity during that period. Waters in the Gulf of Mexico typically have high particulate loads; therefore, once particulate matter is suspended, it represents a viable route of exposure to *P. setiferus* (which is primarily pelagic) and *P. aztecus* (which is both demersal and pelagic over the course of a day). Some participants felt that this information suggests that effluents released from infected farm ponds could represent a viable pathway for exposure to native populations.

A.1.2.2.4 Transport to Processing Facility Subpathway

The breakout group assigned a rating of **Low (Reasonably Certain)** for this pathway, because cases of accidental shrimp escapement by this route have not been documented and are believed to be virtually nonexistent.

A.1.2.2.5 Sediment and Solid Waste Disposal Subpathway

The breakout group assigned a rating of **Low (Reasonably Certain)** for this pathway, assuming that pond dredging activities do not occur within 30 days of disease outbreak. This judgment is based on the relatively short half-lives of viruses in sediments (estimates of viability ranged from 1 to 2 days for WSSV to 30 days for IHHNV) and also on the knowledge that disposal of solid wastes into the ocean is not permitted under U.S. regulation.

A.1.2.2.6 Bait Shrimp Subpathway

This pathway was evaluated by the "Other Pathways" Breakout Group.

A.1.2.3 Colonization Potential for the Aquaculture Pathway

In evaluating the potential for virus colonization, the group concluded that the probability of nonindigenous viruses successfully colonizing and maintaining a population where introduced is **Low (Very Uncertain).** Some breakout group members expressed concern about the rating of **Low** for colonization potential and offered a dissenting opinion. These individuals believe that the rating should be changed to **Medium**, based on information communicated during plenary discussions and the judgment that pond effluent might provide a continuous input of virus to near-coastal systems. Furthermore, they believe that if the aquaculture industry were to expand significantly along the Gulf coast, this potential might more appropriately be rated as **High.**

Nonetheless, the breakout group concluded that the potential for colonization from U.S. aquaculture sources is **Low**, because of the lack of evidence suggesting establishment of viable virus populations in wild U.S. shrimp stocks introduced via the aquaculture pathway and because virus outbreaks in farm ponds have not been correlated with similar outbreaks in local wild stock. This may not be true in other areas of the world, where past practices have involved the "dumping" of entire ponds when outbreaks have occurred. The breakout group recognized, however, that colonization potential is likely to be virus specific and dependent on the specific shrimp species and its life stage susceptibilities.

A.1.2.4 Spread Potential for the Aquaculture Pathway

After considerable discussion, the breakout group was unable to reach consensus on the potential for the spread of viruses once the viruses had colonized. The group ultimately concluded that the potential ranges from **Low (Relatively Uncertain)** to **High (Very Uncertain).**

Workshop participants suggested that stocks of *P. setiferus* in the Atlantic are genetically homogeneous (Mark Frischer, Skidaway Institute of Oceanography, personal communication), as are the northern and southern populations in the Gulf of Mexico (D. Boudreaux, observer, personal communication). Thus, there is the potential for substantial interaction over broad geographic regions, which could promote the spread of viral infection. However, other penaeid species may not be genetically homogeneous.

During its deliberations, the breakout group considered whether experiences with viral disease in aquaculture farms could be extrapolated to field situations. Participants noted that when an outbreak occurs at a facility, viral infection spreads fairly rapidly within individual ponds and can spread beyond the originally infected pond. The mechanisms of transmission between individuals and from pond to pond remain unknown. The breakout group recognized that disease transmission in aquaculture may not be analogous to transmission in wild populations, due to differences in the relative stress experienced by farm shrimp (e.g., crowding, nutrition, predation).

The breakout group agreed that the potential for spread depends in large part on the time course of the disease and the density of shrimp in wild populations (and therefore the rate of individual encounters). For example, low shrimp densities are likely to hinder disease spread, whereas high densities are likely to promote transmission. The breakout group recognized that spread potential is virus specific as well as host dependent. (TSV and IHHNV are thought to have low spread potential, while the spread potential of YHV and WSSV is currently unknown). Additionally, WSSV, when detected in wild stocks in Asia, is distributed over wide geographic areas. This supports the conclusion that viral disease can spread readily from its original locus of colonization. As noted in the JSA Report, other stressors (such as low dissolved oxygen and extreme salinity) are likely to influence the potential for spread of the disease. The mechanisms of virus transmission and infectivity remain major data gaps with respect to spread potential.

A.1.3 CONSEQUENCES OF ESTABLISHMENT OF VIRUSES FROM AQUACULTURE

To assess the consequences of establishment, the breakout group made the assumption that nonindigenous shrimp viruses are established. However, this assumption does not reflect a belief on the part of the breakout group that viruses have indeed been established in U.S. waters.

The breakout group's evaluations focused on the two assessment endpoints articulated in the JSA report: the direct effects on the survival, growth, and reproduction of wild penaeid shrimp populations and the effects on ecological structure and function of marine communities as they affect wild shrimp populations. The breakout group gave primary attention to the first assessment endpoint.

A.1.3.1 Direct Effects on Wild Shrimp Populations

The breakout group concluded that direct effects on wild shrimp populations are **Low to Medium (Very Uncertain).** Participants noted that penaeid shrimp can be characterized as "r-selected" organisms because they display an annual life history pattern with high reproductive output and high mortality during early life stages. In reviewing the existing information, the breakout group concluded that mass mortalities of adult shrimp typically have short-term repercussions on standing shrimp stocks. For example, the suspected 1987 IHHNV-induced mortality event in the Gulf of California (Pantoja-Morales, 1993) was associated with reductions in *P. stylirostris* population abundances for approximately 6 to 7 years, but stocks are reported to be returning to preoutbreak levels. (No specific references were offered in support of this contention, and considerable doubts remain about the role that IHHNV played in the observed population declines.) Additionally, participants noted that because of high fecundity and migratory behavior, *P. setiferus* is capable of rebounding from a very low population size in one year to high abundances in the next year, if environmental conditions are favorable. This has been observed off the South Carolina coast several times in the past 50 years (Linder & Anderson, 1956; McKenzie, 1981). A reported increase in reproductive output of wild shrimp populations in Honduras during the 1994 outbreak of TSV provides additional support for demographic compensatory responses (R. Laramore, observer, personal communication), although it was noted that other factors may have contributed to these population changes. Along with anecdotal information regarding the possible long-term effects of viral infections in Latin American and Asian shrimp populations, the breakout group determined that these observations suggest that direct mortality effects would be relatively transitory. Also, it was suggested that initial outbreaks could lead to enhanced resistance to future viral infection, based on the observation that resistance to IHHNV appears to have increased in all populations tested since the identification of this virus in Hawaiian stocks (Lightner, personal communication).

In addition to direct mortality effects, the breakout group discussed the potential for sublethal effects of viruses on shrimp reproduction and growth. The breakout group was aware of no information describing adverse viral effects on reproductive potential of infected individuals. One expert noted that reproductive output of infected *P. vannamei* brood stock appears to be unaffected by viral infection (F. Jaenike, personal communication). However, in contrast to the previous statement, individual growth impairment in offspring of *P. vannamei* infected with IHHNV has been documented (Fulks & Main, 1992). Assuming that fecundity of female *Penaeus* is an increasing function of size (a phenomenon common in other invertebrate species), breakout group participants considered that stunted growth of offspring could result in reduced reproductive output of the second generation. The breakout group concluded that individual growth impacts could therefore cause population-level effects, although an analysis of the importance of reproduction to shrimp population dynamics would be required to support this conclusion.

To complete its evaluation of direct consequences of viruses to shrimp populations, the breakout group considered a scenario in which a shrimp population experiences a 50 percent decrease in abundance for 5 years as a result of viral outbreak. (This scenario is similar to the Gulf of California situation described by Pantoja-Morales.) By extrapolating from the information summarized previously, the breakout group suggested that the direct consequences on population abundance might be short lived and that stocks would rapidly recover to historic abundances; therefore, the environmental impacts would be low to medium for the immediate population. The breakout group recognized, however, that the genetic consequences of rapid reductions in population abundance (the so-called “founder effect”) are unknown but potentially important. Substantial uncertainty surrounds this rating due to the lack of information regarding analogous situations in actual wild populations and the lack of direct experimental evidence.

A.1.3.2 Effects on Ecological Structure and Function

The breakout group did not rate this element due to insufficient data and a lack of time for a thorough evaluation. The breakout group identified examples in which other invertebrate species have experienced severe disease consequences:

- The near decimation of oysters (*Crassostrea virginica*) by the protozoan pathogens *Haplosporidium nelsoni* and *Perkinsus marinus*, called MSX and dermo disease respectively (Haskin & Andrews, 1988; Andrews, 1996; Burreson & Ragone-Calvo, 1996), has resulted in significant changes in the oyster reef habitat throughout Chesapeake Bay and dramatically reduced the rate at which bay water was filtered by feeding bivalves (Kennedy, 1996).

- Insect/virus associations in which high abundances of the host species promote rapid outbreaks of viral disease, followed by dramatic declines in the host, near-disappearance of the virus, and reestablishment of the host (S. Thiem, personal communication).

- The introduction into Scandinavia of North American crayfish that were carriers of the freshwater crayfish plague *Aphanomyces astaci* (Unestam & Weiss, 1970).

Some breakout group members believed that these examples might serve as models for extrapolating potential consequences of viral establishment in aquatic systems as they affect shrimp populations. These examples may show how ecological systems might be affected by viral outbreaks in shrimp. The breakout group recognized that careful analysis of these examples would be needed to identify similarities and differences relative to the shrimp virus situation.

The aquaculture breakout group did not discuss the effects of viral disease on other components of the ecosystem that might influence dynamics of shrimp populations. Subsequent plenary discussion, however, suggested that other crustaceans (notably paleomonids or “grass shrimp”) might suffer negative impacts with potentially severe consequences to the ecological system as a whole. The breakout group suggested that fish catch data maintained by Mexico during the Gulf of California shrimp decline might help provide insight on possible impacts of shrimp viruses on nonshrimp species.

The breakout group agreed that development of an epidemiological model describing virus-shrimp interactions and subsequent sensitivity analyses of its results would be useful for identifying critical areas of uncertainty and prioritizing research needs. Such a model would permit initial quantitative assessments of the potential consequences of viral infection on wild shrimp populations.

A-2. Report Of The Shrimp Processing Breakout Group

A.2.1 INTRODUCTION

This breakout group was charged with assessing the risk associated with introduction of nonindigenous virus to wild shrimp populations from the shrimp processing pathway (see Figure A-2).

Currently, over 60 countries export both pond-raised and wild shrimp to the United States Over one-half of the shrimp processed in the United States is imported from foreign countries, where viral diseases may be a problem. To minimize disease effects on cultured shrimp yield, some countries harvest shrimp during the early stages of a disease outbreak. This strategy avoids high mortality and catastrophic economic losses, but it increases the likelihood that shrimp imported to the United States will be contaminated with viable viruses (Lightner, 1996a). Shrimp infected with WSSV, YHV, and TSV have been identified in retail stores in the United States (D. Lightner, unpublished); therefore, the importation and processing of infected shrimp may increase the potential for the introduction of pathogenic viruses into coastal waters adjacent to processing plants. This pathway may thus pose a threat to wild shrimp populations (JSA, 1997).

The breakout group reviewed the steps in shrimp processing to identify the potential pathways for the release of virus-contaminated material into the environment. This information was used to examine the conceptual model contained in the JSA report (Figure A-2) to ensure the model's completeness and to evaluate the probability of establishment, impact, and risk for each of the pathways.

The steps in the commercial processing of shrimp are described in Figure A-3. Of the shrimp processed in the United States, 80 percent of total crop is foreign and 20 percent is domestic in origin. Of the imported shrimp, 50 percent is farm raised and 50 percent is wild catch. Most foreign shrimp arrives frozen and generally without heads. Approximately 50 percent of domestic landings arrive at processing plants frozen, and the remainder is fresh. Therefore, only about 10 percent of the total shrimp processed in the United States is actually fresh. The breakout group estimated that up to 40 percent of the total shrimp processed in the United States arrives at processing plants without heads. Because shrimp heads can carry a high concentration of some viruses, the presence or absence of heads on shrimp arriving in the United States is significant.

Processing involves several steps, including thawing (if the shrimp arrive frozen), grading, peeling, and culling (see Figure A-3). Participants noted that no water is transferred when foreign, frozen shrimp arrives in the United States on container ships. Liquid effluent produced from thawing, culling, and washing is either sent to wastewater treatment facilities or is discharged into the coastal environment without treatment. Participants noted that the level of treatment varies according to state requirements. For example, Florida requires treatment of all

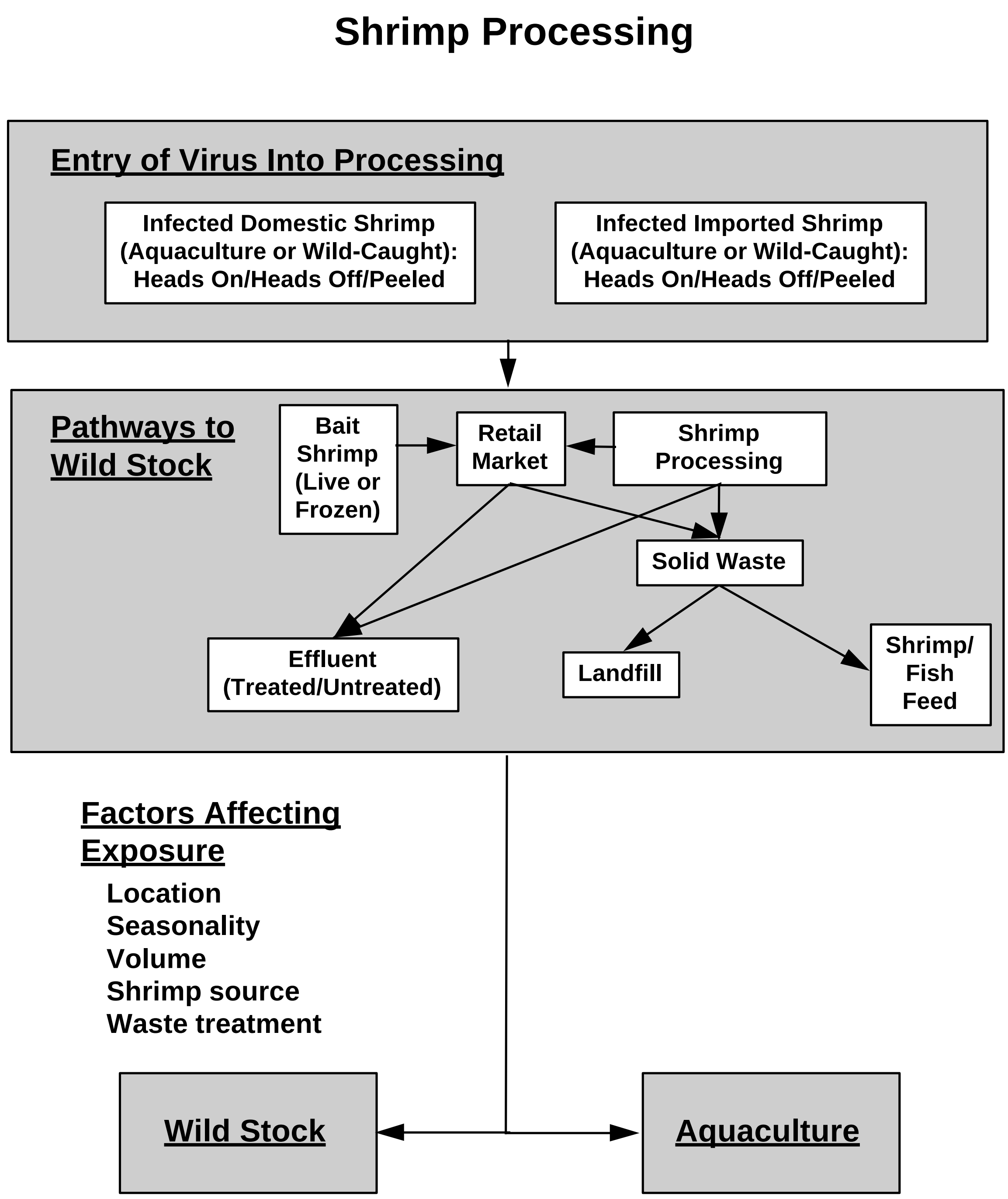

Figure A-2. Conceptual model: Virus sources and pathways for shrimp processing (JSA, 1997)

IQF Cooked Shrimp Production Flow

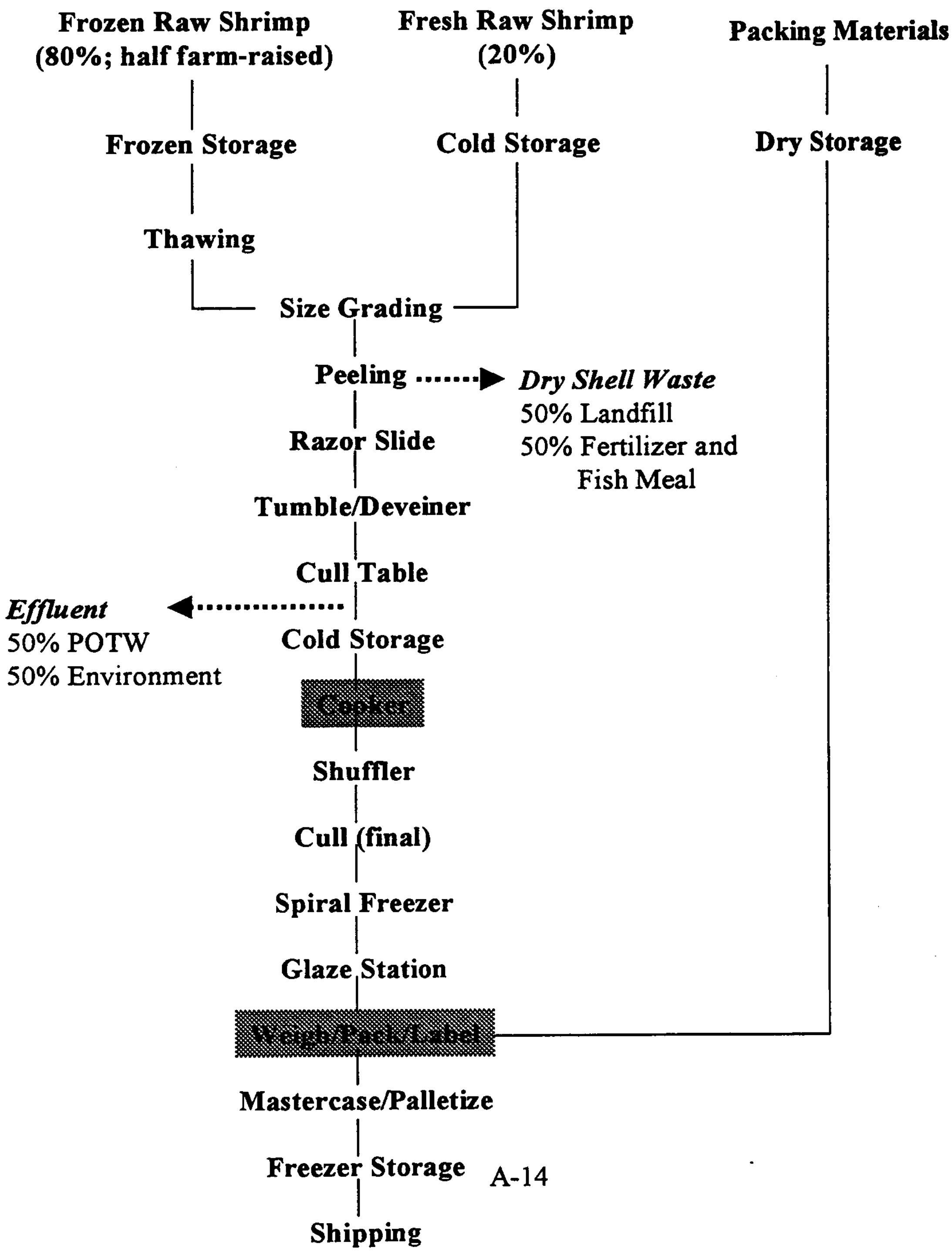

Figure A-3. Flow diagram for shrimp processing.

effluent from shrimp processing. Breakout group members estimated that, nationally, 40 to 50 percent of shrimp-processing effluent is treated. Breakout group members concluded that the discharge of processing effluent from wastewater treatment facilities poses no potential risk because it is believed that the disinfection process is likely to kill viruses. The direct discharge of processing effluent into estuarine waters, however, may represent an important pathway for the establishment of viruses in the environment. Breakout group members felt that this pathway may represent a frequent or continuous source of virus into the environment, thereby increasing the probability of establishment.

Solid waste is generated primarily during peeling, when shrimp shells are removed and sent to landfills or processed for fish feed or fertilizer. Breakout group members noted that, in general, landfills are covered in 24 hours; however, seagulls and land crabs (*Sesarma*) have been reported to immediately descend on shrimp heads once they reach the landfill. There is evidence that TSV can survive intact in seagull feces (D. Lightner, personal communication), thereby providing a potentially important pathway for viruses to contaminate both aquaculture facilities as well as nearshore bays and estuaries.

Processing operations have an effect on the viability of some viruses. For example, breakout group members reported that the viability of WSSV (and YHV, by analogy) declines with increasing frequency of freeze/thaw conditions, but this is not the case for either IHHNV or TSV. This difference in persistence may result from the size and structure of the viruses; IHHNV and TSV are small virus particles whereas WSSV and YHV are larger, more complex viruses that may be more labile (Table A-2). Similarly, breakout group participants noted that experimental evidence shows that IHHNV and TSV have longer half-lives (28 days) in open water than do WSSV and YHV (7 days) (J. Lotz, personal communication, for TSV and WSSV; Flegel et al., 1995, for YHV).

The breakout group noted that effluent from shrimp boats is of minimal concern, because it represents such a small amount of the total potential pathways of virus introduction into the system.

Based on its analysis of shrimp processing, the breakout group decided that the basic elements of the conceptual model presented in the JSA report adequately represent the major pathways associated with processing. For the purposes of this exercise, the breakout group selected four pathways for evaluation: treated effluent, untreated effluent, solid waste in landfills, and shrimp feed/fish feed.

A summary of risk ratings discussed by the shrimp processing breakout group is provided in Table A-3.

Table A-2. Virus persistence, virulence, and infectivity

	IHHNV	TSV	YHV	WSSV
Persistence (1 = least, 4 = most)	**3.5**	**3.5**	**1.5**	**1.5**
Virulence to Gulf Species (1 = least, 4 = most)	1	2	3	4
	Relative Infectivity			
Penaeus setiferus				
Larvae	—	—	ND	ND
Post-larvae	—	++	—	++
Juvenile	+	+	++	++
Adult	ND	+	ND	ND
Penaeus duorarum				
Larvae	—	—	ND	ND
Post-larvae	—	—	—	++
Juvenile	+	+	++	+
Adult	ND	ND	ND	ND
Penaeus aztecus				
Larvae	—	—	ND	ND
Post-larvae	—	+	—	++
Juvenile	+	+	++	+
Adult	ND	ND	ND	

INFECTIVITY

ND = No data

+ = Infectious

++ = Mortality

— = Tried but negative

Table A-3. Summary of shrimp processing breakout group risk rankings

Refer to supporting discussion in the text to properly evaluate information presented in this table. The risk assessment process is described in Section 2.1 and Appendix G.

Probability of Establishment		Pathways to the Environment			
		Treated Effluent	**Untreated Effluent**	**Landfill**	**Shrimp/Fish Feeds**
Association with Pathway		**High/ very certain**	**High/ very certain**	**High/ very certain**	**High/very certain**
Entry Potential		**Low/ very certain**	**High/ very certain**	**Medium/ reasonably certain**	**Low/ very certain**
Colonization Potential		**Low/ very certain**	**Medium/ moderately certain**	**Low/ reasonably uncertain**	**Low/ very certain**
Spread Potential		**Low/ very certain**	**Medium/ moderately certain**	**Low/ reasonably uncertain**	**Low/ very certain**
Overall Probability of Establishment		**Low**	**Medium**	**Low**	**Low**
Consequences of Establishment	**Local**	**Low-medium/ reasonably uncertain**	**Low-medium/ reasonably uncertain**	**Low-medium/ reasonably uncertain**	**Low-medium/ reasonably uncertain**
	Large Scale	**Low/highly uncertain**	**Low/highly uncertain**	**Low/highly uncertain**	**Low/highly uncertain**
Overall Risk Estimate	**Local**	Low-medium	Medium	Low-medium	Low-medium
	Large Scale	Low	Medium	Low	Low

A.2.2 PROBABILITY OF ESTABLISHMENT OF VIRUSES FROM SHRIMP PROCESSING

A.2.2.1 Factors Influencing Colonization and Spread from Shrimp Processing

To accurately assess the probability of establishment of viruses released from shrimp processing, the breakout group reviewed the concepts of colonization/infectivity potential and spread potential, which are two key elements of the establishment process that may ultimately influence environmental impact. Participants noted that implicit in any discussion of risk is the issue of co-occurrence between the stressor (viruses) and the receptor (wild shrimp populations). Exposure to the shrimp virus, therefore, depends not only on the spatial and temporal patterns of viral entry into coastal and marine systems but also on the movements and life-history patterns of the shrimp (JSA, 1997). To help understand this concept, the breakout group discussed the spatial and temporal distribution of the shrimp populations.

A.2.2.1.1 Life-History/Behavior

Breakout group members noted that shrimp populations move into the nearshore regions as postlarvae and as juveniles during the spring. During these life stages, shrimp may be more likely to be exposed to viruses entering from onshore processing discharges or from landfills via avian and crustacean vectors. It was also noted that prior to leaving the estuaries in late summer and early fall, many shrimp undergo a “staging period” in which different species commingle and aggregate in high densities in the nearshore environment for 1 to two months.

The breakout group hypothesized that this behavior increases the likelihood for exposure and subsequent transmission and spread of disease. It was suggested that this hypothesis is probably valid for IHHNV and possibly for WSSV, but not for TSV. A breakout group member also noted that the spread of virus is a function of the different susceptibilities of shrimp species, their life stages (Table A-2), and the ways in which the shrimp are distributed. For example, if shrimp are homogeneously distributed throughout the Gulf, they act as one population. However, participants noted that a more likely scenario is that there are localized areas with high shrimp densities and other areas where there are no shrimp. Even within good habitat, populations are likely to be patchy.

A.2.2.1.2 Population Density

To determine if population density affects disease outcomes, the breakout group discussed whether experiences in aquaculture can be related to field populations. Breakout group members noted that the virus will create a long-term problem in aquaculture when densities are high. For example, in 1996, South Carolina farms experienced widespread infection with TSV. Not all ponds, however, became infected; ponds stocked less densely appeared to avoid the disease. Breakout group members suggested that densely populated conditions create a stressful environment that makes shrimp more susceptible to the spread of disease. This hypothesis is supported by observations from more "natural" impoundments in South Carolina coastal waters, where shrimp densities are reported to be lower and no disease was found.

A.2.2.1.3 Persistence and Virulence

Knowledge of the persistence and virulence of viruses in various environmental media is important to predicting the probability of infection/colonization in wild shrimp populations. Breakout group participants cited data suggesting that persistence in water is virus dependent. IHHNV and TSV persist for weeks to a month, and WSSV and YHV persist for days (Table A-2). Virulence of the four viruses was considered by breakout group participants and ranked in decreasing order as follows: WSSV, YHV, TSV, IHHNV. In addition, there is a wide range of sensitivity among species and among life-history stages within a species. Participants noted that IHHNV, though very persistent, is not particularly virulent to Gulf species. It has only been detected within juveniles and has not been known to cause mortality. WSSV is least persistent but appears to be very virulent (Lightner, 1996), causing mortalities to the postlarvae of all three Gulf species in laboratory experiments (Lightner et al., in press). Variations in persistence, virulence, and life-stage sensitivity underscore the uncertainties associated with determining colonization potential.

A.2.2.1.4 Routes of Infection

To determine the potential for viral establishment, the breakout group also considered the primary routes of infection. There are four plausible pathways: exposure to water (in particular, contact with respiratory surfaces), ingestion of water and associated particles, ingestion of other infected shrimp, and transmission from infected spawning adults through gametes to larvae. It was noted that this last pathway is limited to the offshore stage of the shrimp's life history, while the other three pathways are of greater significance during nearshore stages. Breakout group participants further suggested that animal vectors such as sea gulls and land crabs could represent plausible routes of exposure from solid waste disposal of processed shrimp. It was proposed that sea gulls, which eat potentially infected carcasses disposed at landfills, could disperse virus through their excrement, thereby infecting coastal ponds. Some breakout group members cited reports from Thailand that land crabs (*Sesarma sp.*) feeding on infected matter in landfills could be infected with WSSV and carry the virus back to coastal environments.

A.2.2.1.5 Spatial Scale

Breakout group participants considered that spatial scale is an important factor in both the spread and the probability of environmental impacts. The breakout group generally agreed that local discharges of virus-laden effluents have a reasonable likelihood of infecting a local population of shrimp, particularly in a closed embayment with restricted exchange. Participants hypothesized that such a localized population would be likely to extinguish itself as a result of disease and thus have little or no effect on the population as a whole. Participants noted, however, that there is no evidence to support such a hypothesis.

Similar scenarios can be constructed for large-scale impacts. For example, the "staging" and "aggregating" behavior discussed previously provides an opportunity for a locally infected population to commingle with other species at high densities, thereby increasing the likelihood of transmission and the spread of the virus. Furthermore, the subsequent offshore migration provides a vector for the virus to reach other populations, thereby potentially transmitting viruses through the reproductive cycle. Participants noted that, while scenarios such as these may be plausible, they tend to have very high uncertainty.

A.2.2.2 Pathway Analyses for Shrimp Processing

Breakout group participants observed that several potential exposure pathways can be developed for the conceptual model for shrimp processing. Both shrimp processing plants and retail outlets produce liquid effluent. The proportion of untreated effluent from the retail sector is likely to be relatively unimportant, because most effluent is directed to municipal treatment facilities. Breakout group members recognized that this is not the case in processing, where the volumes of liquid effluent are quite large. The breakout group estimated, however, that at least 50 percent of liquid effluent from processing passes through a municipal treatment facility, which potentially reduces the total risk from this pathway. In addition to effluents, both the retail and processing sectors produce solid waste in the form of shells and heads, which are disposed of either in landfills or used in the production of shrimp or fish feed.

The breakout group qualitatively estimated the probability of establishment of the virus in wild shrimp populations for the following pathways: treated effluent from shrimp processing and retail, untreated effluent from shrimp processing and retail, solid wastes to landfills, and solid wastes to shrimp feed. The breakout group generally agreed that there is a very high probability that wild and farmed foreign shrimp in each of the four pathways are contaminated with viruses. Because foreign shrimp compose 80 percent of the total shrimp consumed in the United States, they represent a major source of potential infection of U.S. farmed and wild shrimp populations. The breakout group therefore agreed that there is a **High** probability of viruses being associated with the pathways leading from both processing and retail to the environment. The breakout group was **Very Certain** of this ranking. These rankings are based on general knowledge**,** with empirical data for the presence of both WSSV and YHV in foreign products.

A.2.2.2.1 Treated Effluent

Because of the high likelihood that virus-infected shrimp may be in this pathway, the primary effluent emanating from plants and retail markets is very likely to carry viruses; however, both retail and processing effluent treated at municipal treatment plants are highly unlikely to retain live viruses because of the rigorous disinfection practices used. As a result, the breakout group was **Very Certain** that the entry potential is **Low**. The breakout group was also **Very Certain** that there was a **Low** risk of colonization and subsequent spread of infection. In this case, the breakout group based its rankings on a general knowledge of virus disinfection and survival in municipal treatment plants and professional judgment regarding its colonization and spread.

A.2.2.2.2 Untreated Effluent

Untreated effluent from retail and, more important, from shrimp processing poses the greatest potential risk for disseminating shrimp viruses to wild shrimp populations. The breakout group estimated that approximately 50 percent of liquid effluent from shrimp processing is untreated and that potentially virus-laden discharges could be released regularly into the environment. The breakout group was **Very Certain** that the probability of the organism surviving in transit and the potential for entry into the environment is **High.**

Breakout group participants noted that the persistence, infectivity, and virulence of the virus in the receiving waters is somewhat more uncertain and is a function of the type of virus, the distance from the receiving waters, the properties of the receiving waters, the stage in the shrimp life cycle, and time of year. Consequently, the breakout group judged the potential for colonization to be **Medium (Moderately Certain).** Because spread of the infection within the wild shrimp population is also dependent on a variety of factors, the breakout group estimated that the potential for spread of the virus once initial colonization has occurred to be **Medium (Moderately Certain).**

A.2.2.2.3 Solid Waste in Landfills

Because of the uncertainties associated with the amount of material reaching landfills, the types of vectors, and the threshold amount of virus required to infect the wild and aquaculture populations, the breakout group found it more difficult to assess the probability of establishment of shrimp virus in the wild population from the solid waste in landfills pathway. The breakout group was **Very Certain** that the shells and particularly the heads of foreign farmed and wild shrimp are highly likely to contain viruses (**High**) and that these viruses are likely to persist for some time in landfill settings. However, the persistence of infectivity of these viruses is unknown.

Participants noted that land crabs (*Sesarma*) and sea gulls are two primary vectors thought to move viruses from the landfills to estuarine waters. Both of these vectors are known to carry viruses. The breakout group also noted that WSSV and YHV are not known to pass through these animals' digestive systems in an infective state; however, TSV is known to pass through the guts of seagulls in an infectious state. At issue is whether the concentrations and frequency of virus introduction from these vectors is sufficient to exceed the threshold level required to infect wild and aquaculture shrimp populations.

An important factor in virus transmission is that the virus is concentrated in the heads (specifically, the lymphoid organ) of shrimp that survive TSV infection. The virus is systemic in the bodies of shrimp at the early stages of TSV and YHV infection. Breakout group members observed that, because shrimp from Asia are being harvested at the onset of infection so that the harvest is not lost, some imported shrimp are now noticeably smaller. The breakout group was **Reasonably Certain** that there is a **Medium** probability of entry potential from landfills to estuaries. Primarily because of the absence of virus-to-shrimp dose-response data and the uncertainties (**Reasonably Uncertain**) associated with frequency and concentration of viruses being introduced by these vectors, the group believed that there is only a **Low** likelihood of colonization within the wild population. Participants noted that dose-response data are critical in defining potential threshold levels for colonization. The breakout group also expressed caution in evaluating the potential for spread or viruses in the wild populations (**Low [Reasonably Uncertain]**).

Although the breakout group did not explicitly discuss the solid waste in landfills pathway in terms of effects to aquaculture, participants hypothesized that there is a greater likelihood of colonization and spread in closed ponds than in open circulating estuaries. Participants noted that there is a higher probability of establishment from repeated small inocula from seagulls and crabs in small ponds than in estuaries. Because of the increased density of organisms in aquaculture systems, breakout group members concluded that the potential for spread is likely to be very high. The critical uncertainties remain (e.g., persistence of virus long enough for wild shrimp to become infective, retention of its virulence, and exceedence of threshold dose). Therefore, the breakout group determined that colonization in aquaculture settings from this pathway is ranked **Medium (Moderately Certain).** However, participants recognized that once the virus has colonized, the probability of spread is ranked as **High** with a fair amount of confidence (**Reasonably Certain**).

A.2.2.2.4 Shrimp and Fish Feeds

One of the important markets for shrimp by-products (e.g., heads and shells) is the shrimp and fish feed processing industry. The breakout group did not have information about the volume of shrimp by-products that contribute to this pathway, but the group was very confident that shrimp by-products can be virus contaminated (**High [Very Certain]).** However, because shrimp and fish feed are processed at very high temperatures, there is little chance that the virus can survive and be a threat to the environment. The breakout group was therefore **Very Confident** that the entry potential of viruses into the environment through this pathway is very **Low.** The group was **Very Certain** that the colonization and spread potentials are **Low.** Overall, the breakout group considered the potential risk of establishment from the shrimp feed/fish feed pathway to be very low to nonexistent with very little uncertainty. The "Other Pathways" Breakout Group also evaluated shrimp feed as a source of virus introduction but came to somewhat different conclusions (see Section 3.2.3).

A.2.3 CONSEQUENCES OF ESTABLISHMENT FROM SHRIMP PROCESSING

The breakout group identified three approaches that could be used to estimate the magnitude and probability of environmental impacts from processing discharges into the environment:

- Field studies that associate virus incidence with disease or effects
- Experimental data that link viruses to biological effects such as mortality, reproduction, and growth
- Modeling studies that explore scenarios of virus exposure

A.2.3.1 Field Evidence for Environmental Impact Potential

The breakout group considered whether field observations have been made on the association and/or co-occurrence of viruses and environmental impacts. It also considered whether empirical data exist that associate viral infection with effects on wild shrimp populations. Workshop participants noted that a crayfish introduced from California to Europe may likely have initiated and served as a carrier to spread the freshwater crayfish plague throughout Scandinavia (Unestam & Weiss, 1970). The Gulf of California shrimp declines described by Pantoja-Morales provide another example (Lightner et al., 1992); however, the population declines were not conclusively demonstrated to result from the virus. There is also evidence of WSSV-like infections in wild populations of shrimp from a South Carolina estuary; however, it is not known how long the virus has been in these waters. Data exist on the South Carolina *P. setiferus* catch during the development of shrimp aquaculture in the state (Figure A-4). These data appear to reflect the natural variability of the populations. This variability is largely related to annual spawn success, which is controlled, at least in part, by winter temperatures. Participants emphasized that there is no evidence to suggest that WSSV has affected wild shrimp populations in South Carolina or anywhere in the world. Despite a serious outbreak of TSV in South Carolina in 1996, the 1996 and 1997 crop harvests were near or above the historical mean (Figure A-4). *Baculovirus penaei* (BP) has also been detected in the mysis stage of brown shrimp in the Gulf of Mexico, which suggests that the virus may have been transmitted via gametes from infected parent stock that spawned in open Gulf waters. However, one workshop participant noted that there is no evidence in the literature to suggest that BP can be transmitted via gametes.

Studies of viral infections in populations of shrimp in Honduras suggest that endemic virus has not had an impact on population levels (Laramore, observer comment, also in JSA, 1997).

Finally, workshop participants noted that there is evidence that IHHNV has become established in aquaculture and that stunted growth in *P. vannamei* has occurred. These data suggest that some viruses (e.g., IHHNV and TSV) exist in wild populations of shrimp; however, there is currently no evidence (based on shrimp landings) that these infections have caused or are causing impacts.

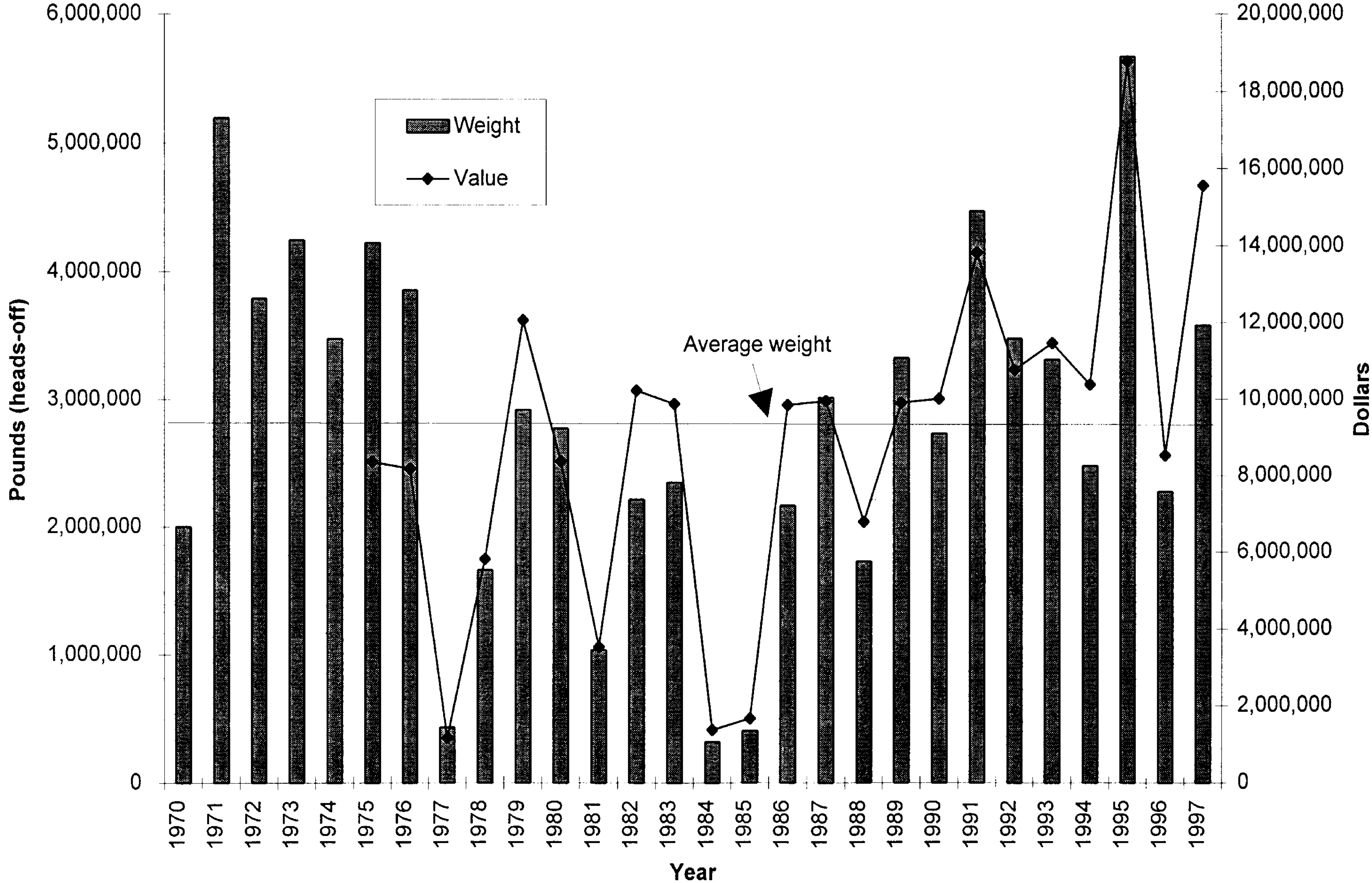

Figure A-4
South Carolina Commercial White Shrimp Landings and Values
(South Carolina Department of Natural Resources, 1998)

It should also be noted that shrimp landings do not necessarily correlate well with shrimp reproduction. The breakout group identified several remaining questions:

- Are chronically infected wild populations at greater risk to challenge from other stressors?
- Is there a delayed expression of chronic viral infection to the populations?
- Have these populations developed resistance to the virus?
- Has the virulence of the virus attenuated so that an equilibrium has been established between virus and host?

A.2.3.2 Experimental Data for Environmental Impacts

Workshop participants noted that laboratory or field experimental data provides another line of evidence for determining the probability of environmental impacts from virus infection of shrimp populations. Studies in aquaculture facilities indicate that virus exposure, infection, and mortality are strongly associated. However, no experimental studies have been conducted on any species that can be used to establish any of the following:

- Dose-response relationships
- Virus transmission rates
- Virus-induced impairment of reproduction
- Virus infection rates
- Transmission between life history stages or species

Therefore, breakout group members concluded that the lack of threshold information makes it impossible to develop infection/colonization estimates with any degree of certainty.

A.2.3.3 Population Modeling

The breakout group briefly discussed the potential use of shrimp population models to estimate impacts from various virus-induced mortality or reproductive impairment scenarios. Because of the commercial importance of shrimp, participants believed that it is highly likely that population models exist for these species. Additionally, participants felt that a large body of catch statistics could be subjected to time series analysis in concert with known periods of virus outbreaks. These types of data may be available for foreign fisheries as well. A modeling framework could be created to examine specific hypotheses by using population, transport, and fate models that incorporate appropriate constants for infection and transmission. Sensitivity analyses could then be performed to determine which parameters are most important and contribute the most uncertainty. Participants concluded that research could then be directed to reduce uncertainty.

A.2.3.4 Summary

A.2.3.4.1 Local Impacts

The breakout group determined that there is a **Low** to **Medium** probability that local impacts will occur from the discharge of untreated liquid effluents from processing plants discharging into coastal waters. The breakout group assigned a medium ranking because of the large amount (e.g., one-half million pounds per day) of contaminated foreign farm-raised shrimp that are routinely processed with untreated effluents (Dunkelberger, personal communication). Sources of uncertainty in this assessment include the virulence and persistence of the virus and the susceptibility of the life stage of the host species. As a result, the breakout group was **Reasonably Uncertain** about the likelihood that local impacts would occur. Furthermore, participants concluded that the infrequency of local impacts to wild shrimp populations supports a **Low** to **Medium** rating for impact.

A.2.3.4.2 Large-Scale Impacts

The breakout group determined that, for several reasons, it is more problematic to estimate the consequence of establishment of virus diseases at large scales than at local scales. In addition to the sources of uncertainty described for local impacts, mechanisms are required to explain a broad-scale transmission of the virus. Breakout group members noted that, while the pre-migration “staging” behavior could serve as a plausible mechanism, its validity has not been demonstrated. The breakout group concluded that there is a **Low** probability of widespread impacts from viral disease in shrimp, but they were **Highly Uncertain** about this rating. To date, however, no evidence from field studies or catch statistics suggests large scale impacts to wild shrimp populations from virus infection.

A-3. Report of the "Other Pathways" Breakout Group

A.3.1 INTRODUCTION

The "Other Pathways" breakout group was charged with assessing the risk associated with introduction of nonindigenous virus to wild shrimp populations from pathways other than shrimp aquaculture or shrimp processing operations. The group first itemized potential pathways and then placed them in two categories: likely pathways and secondary or incidental pathways.

Likely pathways were identified as the following:

- Ballast water
- Bait shrimp
- Shrimp feed
- Animal vectors

Secondary or incidental pathways included:

- Natural spread
- Research and display facilities
- Human sewage
- Fishing vessels
- Hobby and ornamental displays
- Live seafood distribution
- Other crustacean aquaculture
- Incidental introductions

The group also discussed transplantation of wild shrimp from one location to another as a potential source of viruses, but this pathway was dismissed because such activity is illegal in all southeastern Atlantic and Gulf Coast states.

A.3.2 PROBABILITY OF ESTABLISHMENT—LIKELY PATHWAYS

The breakout group discussed and rated, using a qualitative approach, the four likely pathways for their probability of establishment. In addition to compiling their ratings, this breakout group also noted whether their supporting information came from general knowledge, judgmental evaluation, extrapolation, or cited literature (see pp. 22–24, Appendix G). Because time was limited, individual breakout group members rated the secondary or incidental pathways individually, without group discussion. A summary of risk ratings discussed by the "Other Pathways" Breakout Group for likely pathways is provided in Table A-4.

A.3.2.1 Ballast Water

Following the ANSTF approach, the breakout group estimated the probability of the organism being on, with, or in the pathway to be **High** (**Moderately Certain;** professional judgment). The breakout group defined the ballast water pathway to include the water itself, free virus in the water, invertebrate organisms that might or might not carry the virus (either alive or dead), and viruses associated with inorganic particulate material in the water. The breakout group considered that ballast water is used on very large container ships and oil tankers and that therefore discharges from these vessels represent a large volume to the nearshore or offshore environments. The breakout group noted that no one has ever investigated whether ballast water or any of its components contain shrimp viruses. Nonetheless, it is known that many large organisms are discharged routinely with ballast water (e.g., Carlton & Geller, 1993; Williams et al., 1988). These include species of mysid shrimp, some of which have colonized bays and estuaries with devastating effects, and the zebra mussel, which has recently colonized the Great Lakes after frequent discharges in ballast water over an extended period.

The breakout group estimated the probability of the organism surviving in transit in ballast water to be **High** (**Very Certain;** extrapolation from other organisms). Participants concluded that many other organisms are known to survive transit in ballast water, so there is every reason to believe that shrimp viruses could do so as well.

The breakout group estimated the probability of the organism successfully colonizing and maintaining a population where introduced to be **Low** (**Moderately Certain;** extrapolation from

Table A-4. Summary of other pathways breakout group risk rankings for likely pathways to the environment

Refer to supporting discussion in the text to properly evaluate information presented in this table. The risk assessment process is described in Section 2.1 and Appendix G.

Probability of Establishment	Ballast Water	Bait Shrimp		Shrimp Feed		Animal Vectors
		Foreign	Domestic	No Heat	Heat-Treated	
Association with Pathway	High/moderately certain	High/ moderately certain	Low/very certain	Medium/ moderately certain	Medium/ moderately certain	High/very or reasonably certain[1]
Entry Potential	High/very certain	High/very certain	High/very certain	High/ very certain	Low/ very certain	High/reasonably certain
Colonization Potential	Low/moderately certain	High/very uncertain	High/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium to high/ relatively uncertain
Spread Potential	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain
Overall Probability of Establishment	Low	Medium	Low	Medium	Low	Medium

[1] Very certain for gulls and freshwater and marine invertebrates; reasonably certain for other vertebrates.

other organisms). Breakout group participants noted that many organisms are introduced into exotic environments but few survive to colonize. For example, the group noted that only after 70 years of ballast water introductions did the zebra mussel successfully establish itself in the Great Lakes. For penaeid shrimp, however, colonization potential of virus discharged with ballast water will depend on whether the discharge occurs in the open ocean or in nearshore estuarine environments and on contact of the discharges with shrimp. Breakout group members recognized that neither the transmission rates of viruses in open oceans nor the infectivity of the viruses to wild populations is known; only information about laboratory infectivity rates is currently available. A breakout group member provided one example: John Couch, using a baculovirus model, had great difficulty in getting infections to transmit among shrimp. Field surveys of wild shrimp populations in Texas suggest that colonization potential is not high. Studies for the past 25 years on shrimp and other crustacean species have not revealed any new species that have colonized as a result of ballast water discharges. However, the breakout group noted that the volume of ballast water discharged into the Gulf of Mexico along the Texas and Louisiana coasts is low compared to levels discharged into California or the Great Lakes.

The breakout group estimated the probability of the organism to spread beyond the colonized area to be **Medium** (**Very Uncertain;** professional judgment). They believed that the virus could be spread from a small focus of live shrimp that feed on dead infected shrimp discharged with the ballast water. The spread from that focus is dependent on the infectivity threshold of the virus, the transmission rate, and the density of susceptible host species. Breakout group participants determined that each of these factors is dependent on the specific virus and may also be dependent on life stage.

The breakout group concluded that the overall probability of establishment by the ballast water route is **Low** because of the low colonization potential.

A.3.2.2 Bait Shrimp

The breakout group estimated the probability of the organism being on, with, or in the bait shrimp pathway as **High** (**Moderately Certain;** general knowledge) for foreign (frozen) shrimp, and **Low** (**Very Certain;** general knowledge) for domestic (live) shrimp. Anglers use shrimp as bait when fishing for species that naturally eat shrimp. They purchase bait from bait shops or they use shrimp sold in grocery stores for human consumption. It was noted that bait shrimp generally are smaller than those sold for human consumption and are considered substandard. It was suggested that they may originate from aquaculture facilities that have harvested their shrimp prior to full growout because of a viral outbreak. Breakout group participants noted that Latin American and Asian producers may freeze these small shrimp and ship them to the United States for sale as bait, while the larger, uninfected shrimp will be sold at premium prices for human consumption. Therefore, there is a high probability that these smaller, frozen shrimp may contain virus.

Some states (e.g., South Carolina) do not allow the use of nonnative farm shrimp as bait, but domestic aquaculture shrimp may be harvested and sold as live bait. Breakout group participants said that, although it is known that these domestic shrimp carry indigenous viruses (e.g., BP, another baculovirus), there is no evidence to date that these shrimp carry nonindigenous viruses such as those considered by the workshop. Participants noted that domestic shrimp harvested early because of virus problems are likely to be frozen, so there is a low probability that live domestic shrimp bait carry nonindigenous viruses.

The breakout group estimated the probability of the organism surviving in transit to be **High** (**Very Certain;** general knowledge). Participants based this determination on the knowledge that shrimp viruses would be carried in shrimp tissues. It is not likely that the freezing process will significantly reduce the virulence and infectivity of the virus. Instead this may be virus specific.

The breakout group estimated the probability of viruses from bait shrimp successfully colonizing and maintaining a population where introduced to be **High** (**Very Uncertain;** professional judgment). Breakout group members recognized that bait shrimp are deposited in areas where native shrimp are known to occur. Anglers fish in these spots because there is a greater likelihood of catching shrimp-feeding fish in such areas. Therefore, participants noted that the virus has a greater potential to be placed directly into a viable shrimp population. The greatest potential for colonization occurs when an angler disposes of leftover bait by dumping all remaining bait shrimp overboard and into the estuary. These shrimp will sink to the bottom and may be eaten by the native shrimp, thereby creating a direct exposure route.

The breakout group estimated the probability of viruses from bait shrimp to spread beyond the colonized area to be **Medium** (**Very Uncertain;** professional judgment). The virus could be spread from a small focus of shrimp feeding on discarded and infected dead shrimp. As with ballast water discharges, participants noted that the spread from this focus depends on the infectivity threshold of the virus, the transmission rate, and the density of susceptible host species. Each of these factors is dependent on the specific virus and may also depend on shrimp life stage.

The breakout group estimated the overall probability of establishment by the bait shrimp route to be **Medium** for imported foreign frozen bait shrimp and **Low** for domestic bait shrimp.

A.3.2.3 Shrimp Feed

The breakout group estimated the probability of the organism being on, with, or in the shrimp feed pathway as **Medium** (**Moderately Certain;** professional judgment). Shrimp feed is made from soy protein, fish protein (including anchovies and menhaden), shrimp heads, and other types of shrimp and crustaceans (e.g., *Artemia*). The breakout group agreed that some shrimp parts have a high probability of carrying viruses.

The breakout group estimated the probability of the organism surviving in transit as **Low to High** (**Very Certain;** extrapolation from other organisms). The probability of survival in transit depends on whether or not the feed meal is heat treated to a temperature sufficient to kill all viruses. Participants noted that some of the viruses (e.g., TSV) may survive and maintain infectivity even when heated to temperatures greater than 100 °C. While most of the fish meal produced in the United States is subjected to heat treatment that appears to be sufficient to kill the viruses, it is not known for certain that this is the case. Furthermore, workshop participants stated that other countries, such as Mexico, do not heat their meal. The breakout group determined that transit survival probability is **Low** (for heat treated) to **High** (for no treatment).

The breakout group estimated that the probability of the organism successfully colonizing and maintaining a population where introduced as a result of this pathway to be **Medium** (**Very Uncertain;** professional judgment). Breakout group participants noted that virus may be introduced into the environment either through use of the feed in aquaculture or through chumming, which is the dumping of feed into the marine environment to attract other shrimp or fish for easy harvest. The group estimated the risk from chumming to be **Medium** (assuming that live virus is present), because relatively large quantities of material could be dumped within a small area.

The breakout group estimated the probability of the organism to spread beyond the colonized area to be **Medium** (**Very Uncertain;** professional judgment). The spread of virus from the focus of introduction depends on the infectivity threshold of the virus, the transmission rate, and the density of susceptible host species. Each of these factors is dependent on the specific virus and may also depend on shrimp life stage.

As a result of their discussions, the breakout group estimated the overall probability of establishment by the shrimp feed route to be **Medium** to **Low** (depending on whether heat treatment is successful or not).

A.3.2.4 Animal Vectors

The breakout group estimated the probability of the organism being on, with, or in the animal vectors pathway to be **High** (**Very Certain;** published data) for gulls and freshwater and marine invertebrates and **High** (**Reasonably Certain;** extrapolation from other organisms) for other vertebrates. Published data indicate that TSV in shrimp consumed by gulls can be passed through the digestive tract and discharged in fecal matter. Participants noted that gulls and other scavengers (e.g., raccoons) are often seen feeding on dead shrimp and other organic matter associated with aquaculture facilities that have undergone a viral outbreak. Other data demonstrate that water boatmen (Corixids) may pick up virus from aquaculture ponds and then move to nearby natural bodies of water. It was also noted that the viruses WSSV and YHV are carried (as silent carriers, with no infection) by marine invertebrate species in Asia.

The breakout group estimated the probability of the organism surviving in transit to be **High (Reasonably Certain).** Published data have shown that these viruses can survive transmission by at least some of the pathways described previously. Survival may be virus specific, because avian guts have low pH and relatively high temperatures that could inactivate some viruses.
The breakout group estimated the probability of the organism successfully colonizing and maintaining a population where introduced to be **Medium** to **High** (**Relatively Uncertain**;

professional judgment). As detritivores, shrimp are likely to feed on bird fecal matter. Participants observed that the potential for colonization would increase in areas where vector density is high (e.g., when a shrimp die-off occurs in an aquaculture facility, particularly if the facility is near an area that supports wild shrimp populations). Breakout group members noted that genetic variability of shrimp in Asia varies among regions. Areas with less genetic variability may be more susceptible to disease.

The breakout group estimated the probability of the organism to spread beyond the colonized area to be **Medium** (**Very Uncertain;** professional judgment). The virus could be spread from a small focus of infected shrimp. Breakout group members acknowledged that the spread from that focus depends on the infectivity threshold of the virus, the transmission rate, and the density of susceptible host species. In addition, these factors are very dependent on the specific virus and may also depend on shrimp life stage.

The breakout group estimated the overall probability of establishment by the vector route to be **Medium,** depending on the density of vectors and their proximity to wild populations of shrimp or the genetic diversity of the shrimp.

A.3.3 PROBABILITY OF ESTABLISHMENT—SECONDARY OR INCIDENTAL PATHWAYS

Due to time constraints, secondary or incidental pathways were not discussed during the breakout group meeting. Instead, breakout group members rated these pathways individually, using worksheets. No discussion was recorded, and any comments reflect those written on the individual participant’s worksheets. A summary of risk ratings developed by the “Other Pathways” breakout group for secondary or incidental pathways is provided in Table A-5.

A.3.3.1 Natural Spread

Estimate the probability of the organism being on, with, or in the pathway: **Medium** (**Very Uncertain;** professional judgment). This pathway includes the spread of virus from one shrimp population in the Gulf of Mexico to other native populations through natural means, such as movement of infected shrimp or movement of viruses by hurricanes or currents.

Estimate the probability of organism surviving in transit: **High** (**Very Uncertain;** professional judgment).

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: High (**Very Uncertain;** professional judgment).

A.3.3.2 Research and Display Facilities

Estimate the probability of the organism being on, with, or in the pathway: **High** (**Very Certain**; published data); **Low** (**Moderately Certain**; professional judgment); **High (Very Uncertain**; professional judgment). Inoculum to the environment would usually be very small. Research facilities tend to take greater biosecurity precautions than many commercial ones.

Estimate the probability of the organism surviving in transit: **High** (**Very Certain**; published data); **High** (**Moderately Certain**; professional judgment); **High** (**Very Uncertain**; professional judgment).

Estimate the probability of the organism to spread beyond the colonized area: **High (Very Uncertain**; professional judgment).

The overall probability of establishment through natural spread is estimated to be **Medium.**

Table A-5. Summary of other pathways breakout group risk rankings for secondary or incidental pathways to the environment

Refer to supporting discussion in the text to properly evaluate information presented in this table. These pathways were rated individually by breakout group members, and there was no group discussion of these ratings. Consequences of establishment were not rated for these pathways. The risk assessment process is described in Section 2.1 and Appendix G.

Probability of Establishment	Natural Spread	Research and Display Facilities	Human Sewage	Fishing Vessels	Hobby and Ornamental Displays	Live Seafood Distribution	Other Crustacean Aquaculture	Incidental Introductions
Association with Pathway	Medium/ very uncertain	Low/ moderately certain to high/very certain	Medium/ very uncertain	Low to medium/ moderately certain	Low/ moderately certain	Low/ reasonably uncertain	Low/very uncertain to medium/ moderately certain	Low/very uncertain
Entry Potential	High/very uncertain	High/ moderately to very certain	Medium/ very uncertain	High/ reasonably certain	High/ moderately certain	High/ moderately certain	Low/very uncertain to medium/ reasonably certain	Low/very uncertain
Colonization Potential	High/ very uncertain	Low/very certain to high/very uncertain	Medium/ very uncertain	Medium/ reasonably uncertain	Low/ moderately certain	Low/ reasonably uncertain	Low/very uncertain to medium/very uncertain	Low/very uncertain

Table A-5. Summary of other pathways breakout group risk rankings for secondary or incidental pathways to the environment (continued)

Spread Potential	High/ very uncertain	Low/ relatively certain to high/very uncertain	Medium/ very uncertain	Medium/ very uncertain	Medium/very uncertain	Medium/very uncertain	Low/very uncertain to medium/very uncertain	Low/very uncertain
Overall Probability of Establishment	Medium	Low to medium	Medium	Low to medium	Low	Low	Low to medium	Low

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: **Low** (**Very Certain**; published data); **Low** (**Moderately Certain to Very Uncertain;** professional judgment)**; Medium to High** (**Very Uncertain**; professional judgment). This estimate assumes that the research facility is working with organisms that have not been tested to ensure they are Specific Pathogen-Free (SPF) before introduction to the lab. For labs that are specifically involved in research on SPF organisms, the probability would be rated as low.

Estimate probability of organism to spread beyond the colonized area: **Low** (**Relatively Certain;** general knowledge); **Medium** (**Very Certain to Very Uncertain;** professional judgment); **Medium to High** (**Very Uncertain;** professional judgment).

The overall probability of establishment through research and display facilities is estimated to be **Low to Medium.**

A.3.2.3 Human Sewage

Estimate the probability of the organism being on, with, or in the pathway: **Medium** (**Very Uncertain;** professional judgment).

Estimate the probability of the organism surviving in transit: **Medium** (**Very Uncertain;** professional judgment).

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: **Medium** (**Very Uncertain;** professional judgment).

Estimate probability of organism to spread beyond the colonized area: **Medium (Very Uncertain;** professional judgment).

The overall probability of establishment through human sewage is estimated to be **Medium.**

A.3.2.4 Fishing Vessels

Estimate the probability of the organism being on, with, or in the pathway: **Low to Medium** (**Moderately Certain;** professional judgment).

Estimate the probability of the organism surviving in transit: **High (Reasonably Certain;** professional judgment).

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: **Medium** (**Reasonably Uncertain;** professional judgment).

Estimate probability of organism to spread beyond the colonized area: **Medium** (**Very Uncertain;** professional judgment).

The overall probability of establishment through fishing vessels is estimated to be **Low to Medium.**

A.3.2.5 Hobby and Ornamental Displays

Estimate the probability of the organism being on, with, or in the pathway: **Low** (**Moderately Certain;** professional judgment).

Estimate the probability of the organism surviving in transit: **High** (**Moderately Certain;** professional judgment).

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: **Low** (**Moderately Certain;** professional judgment).

Estimate probability of organism to spread beyond the colonized area: **Medium** (**Very Uncertain;** professional judgment).

The overall probability of establishment through hobby and ornamental displays is estimated to be **Low.**

A.3.2.6 Live Seafood Distribution

Estimate the probability of the organism being on, with, or in the pathway: **Low** (**Reasonably Uncertain;** professional judgment). There is very little live seafood imported into the United States.

Estimate the probability of the organism surviving in transit: **High** (**Moderately Certain;** professional judgment).

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: **Low** (**Reasonably Uncertain;** professional judgment).

Estimate probability of organism to spread beyond the colonized area: **Medium** (**Very Uncertain;** professional judgment).

The overall probability of establishment through live seafood distribution is estimated to be **Low.**

A.3.2.7 Other Crustacean Aquaculture

Estimate the probability of the organism being on, with, or in the pathway: **Low** (**Very Uncertain**; professional judgment); **Medium** (**Moderately Certain**; professional judgment); **Low** (**Very Uncertain**; professional judgment).

Estimate the probability of the organism surviving in transit: **Low** (**Very Uncertain;** professional judgment); **Medium** (**Reasonably Certain**; professional judgment); **Low** (**Very Uncertain;** professional judgment).

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: **Low** (**Very Uncertain;** professional judgment); **Low (Reasonably Certain;** professional judgment); **Medium** (**Very Uncertain;** professional judgment). Crayfish are freshwater species and crayfish farms are not as close to coastal waters as shrimp farms.

Estimate probability of organism to spread beyond the colonized area: **Low** (**Very Uncertain;** professional judgment); **Medium** (**Very Uncertain;** professional judgment); **Low** (**Very Uncertain;** professional judgment).

The overall probability of establishment through other crustacean aquaculture is estimated to be **Low to Medium.**

A.3.2.8 Incidental Introductions

Estimate the probability of the organism being on, with, or in the pathway: **Low** (**Very Uncertain;** professional judgment).

Estimate the probability of the organism surviving in transit: **Low** (**Very Uncertain;** professional judgment).

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: **Low** (**Very Uncertain;** professional judgment).

Estimate probability of organism to spread beyond the colonized area: **Low** (**Very Uncertain;** professional judgment).

The overall probability of establishment through incidental introductions is estimated to be **Low.**

A.3.4 CONSEQUENCES OF ESTABLISHMENT IN "OTHER PATHWAYS"

To begin its discussions of the consequences of establishment, the breakout group was presented with the assumption that it is difficult to start an epizootic but eventually one will occur, given continued input of virus to the estuarine or marine environments.
The group agreed that this basic premise is valid, although some members preferred to say that an epizootic "might" rather than "will" occur. The breakout group noted that it is very difficult to infect animals, even in laboratory settings. In some studies, attempts to infect *P. vannamei*

postlarvae with WSSV by feeding resulted in 100 percent survival (Overstreet et al., 1997). Many factors influence the susceptibility of shrimp to experimental virus infection, including host species, the manner in which the virus is prepared and stored, and environmental conditions in which the shrimp are maintained. In addition, an infected shrimp may or may not exhibit clinical signs of infection and may or may not die from the disease. The group briefly discussed which of the four viruses of concern would be most likely to cause a natural epidemic. The group thought that WSSV and YHV are more likely than IHHNV or TSV to cause acute mortality but that IHHNV and TSV are more likely to become endemic.

A breakout group member stated that genetic resistance is likely to differ among populations. Without further knowledge of this variability among Gulf Coast shrimp, for example, it is difficult to make accurate predictions about which area has the highest potential for an epizootic. An individual also noted that a published paper from Thailand shows that southern populations of shrimp are much less genetically diverse than those from the northern part of the country. Participants noted that it has been hypothesized that these differences are due to release of shrimp from aquaculture into the wild.

Breakout group members observed that if a virus is successfully introduced into an estuary and wipes out the entire local shrimp population, the effects are likely to be short-term. They noted that repopulation could occur in 3 to 5 years, or perhaps sooner (see Figure A-4). This estimate is based purely on professional judgment and not on any hard data. Similar population impacts and recoveries have been observed from natural stressors such as low temperatures or freshwater flooding. Breakout group members pointed out that recovery from winter kills may occur within 1 year (Figure A-4). The group indicated that information is needed to better determine whether the shrimp that recolonize an area differ genetically from the original stock.

The breakout group also discussed the shrimp-virus interaction, noting that the target organ of the virus may influence its infectivity and be dependent on the life stage of the shrimp. For example, juvenile shrimp have a larger gut-to-body mass ratio than older shrimp and are therefore more susceptible to the viruses (such as TSV) that replicate in gut epithelium. Participants recognized that much more information is needed on the shrimp immune response to viral infection. Some noted that viruses are typically able to escape cellular immune mechanisms such as hemocytes or macrophages, by moving from cell to cell rather than through the hemolymph. Participants concluded that coupling the understanding of target-organ sensitivity with information about resistance will improve the ability to predict which shrimp are likely to become carriers. Virus carriers may have active infections (perhaps systemic) and continuously shed virus, or they might be silent carriers with the virus sequestered in particular organs and expressed only during times of stress.

The breakout group also discussed whether a shrimp population would develop tolerance following a major virus disease outbreak. If the population were to develop tolerance, the virus could remain endemic in the population, and disease outbreaks could occur cyclically. Population numbers may be stable but at a lower level than would be present in the absence of the virus. One individual suggested that information on wild shrimp population levels before and after the introduction of TSV into Honduras and Ecuador may provide insight into this hypothesis.

The breakout group briefly discussed cross-species transmission (shrimp-to-shrimp or shrimp-to-other-crustacea) and speculated that virulence may change during such a passage. Some evidence exists that these viruses can replicate in crabs or other shrimp without causing disease symptoms; however, it is unknown whether this would increase or decrease virulence, although one individual pointed out that all viruses change genetically over time.

The breakout group noted that it would be very difficult to diagnose the cause of a decline in a population of shrimp because many factors interact to cause natural population fluctuations of up to 25 percent per year. They concluded that identification of virus in the shrimp would indicate that the virus may have played a part in the change, but it would not establish a cause-and-effect relationship.

The potential impact of viruses on the entire shrimp population is unknown. Some participants suggested that natural mortality rates in shrimp approach 100 percent and that approximately 90 percent of the shrimp are harvested before they die. Virus-induced mortality, therefore, should not be biologically significant. Virus-induced mortality, however, may have economic significance if the shrimp are killed before reaching harvestable size. One breakout group member pointed out that although the mortality in the postlarval shrimp that leave the estuary is naturally high, it must be less than 100 percent or there would be no shrimp left to reproduce. Participants suggested that complete mortality of a single estuary’s shrimp (which may occur following a virus outbreak) may not have a significant impact on the overall population. Recolonization of the estuary would occur as shrimp from nearby locations drift in on currents in subsequent years; however, as stated previously, recolonization may take from 3 to 5 years (or less if the population responds in a similar fashion as it does to natural stressors, such as temperature). The breakout group concluded that, in the short term, the alteration of the estuarine ecosystem could be substantial.

The breakout group also discussed the potential for viruses to affect estuarine ecology by infecting other species of shrimp, such as grass shrimp. Participants noted that grass shrimp (*Paleomonetes sp.*) are an important part of the estuarine food web. Many species of fish (and penaeid shrimp) rely on grass shrimp as an important prey item. Data from Thailand suggest that grass shrimp may be carriers of one or more of these viruses, but data on infectivity rates and effects are lacking.

The breakout group acknowledged that an important area of uncertainty is whether viruses that are endemic in shrimp populations have the potential to change the population's reproduction rate. A change in the reproduction rate could occur either by directly affecting the number or viability of gametes produced or by reducing growth and subsequent reproduction of offspring of infected individuals. Without this information, the breakout group concluded that it will not be possible to make any statements about population consequences beyond the educated guesses outlined previously.

A.3.5 RESEARCH NEEDS IDENTIFIED BY THE "OTHER PATHWAYS" BREAKOUT GROUP

The breakout group identified the following important research needs:

- Tests for virus identification are critical. Tests must have specificity for the virus and be standardized across labs. Tests should be useable on different shrimp species, live shrimp, dead shrimp (frozen or fresh), and pieces of shrimp tissue.

- Tests for infectivity are needed to establish the threshold number of viruses that would be required for colonization potential. At least two tests such as a PCR and ELISA or a PCR and a bioassay, should be employed. Natural susceptibility of native shrimp to nonindigenous viruses needs to be documented better, including looking for differences among genetic strains or within populations with more or less genetic diversity.

- Virus inactivation parameters should be better identified. The amount of duration of heat treatment for reactivation of the various viruses should be studied systematically. Also, other environmental factors that could inactivate the virus (e.g., dryness and ultraviolet light) should be elucidated to understand how long a virus can persist outside its host.

- A map of the known locations around the world of virus prevalence in shrimp should be created so that potential sources can be identified. Because general surveys have not been done widely, areas in which the virus is not identified as prevalent may or may not be infected.

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