

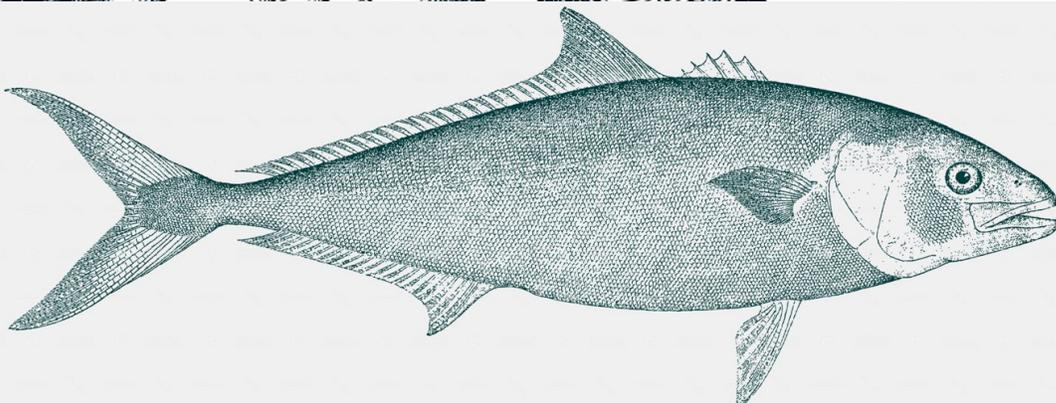


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Review of Best Practices for Biosecurity and Disease Management for Marine Aquaculture in U.S. Waters



February 2023

U.S. DEPARTMENT OF COMMERCE

National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northwest Fisheries Science Center

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Review of Best Practices for Biosecurity and Disease Management for Marine Aquaculture in U.S. Waters

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<https://doi.org/10.25923/b4qp-9e65>

February 2023

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U.S. DEPARTMENT OF COMMERCE

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Introduction:

(left) Wellfleet Oyster Farm, Massachusetts. Photograph by A. Michaelis, NOAA Fisheries. <https://media.fisheries.noaa.gov/styles/original/s3/2022-09/wellfleet-oyster-farm-noaa-750x500.png?itok=ZZdiSgGy>

(right) Seagrove Kelp Company farm in Doyle Bay, Alaska. Photograph by NOAA Fisheries. <https://media.fisheries.noaa.gov/styles/original/s3/2022-09/seagrove-kelp-co-harvest-alaska-fisheries-science-center-2021.JPG?itok=bwoH13SK>

Disease Management and Biosecurity Across Sectors:

(left) Large-scale culture of microalgae for larval shellfish food. Photograph by L. Cresswell, Southern Regional Aquaculture Center. <https://shellfish.ifas.ufl.edu/wp-content/uploads/Phytoplankton-Culture-for-Aquaculture-Feed.pdf>

(right) Rearing tanks for juvenile fish. Photograph by L. Rhodes, NOAA Fisheries.

Finfish-Specific Biosecurity:

(left) Striped bass. Photograph by R. Hagerty, U.S. Fish and Wildlife Service. <https://www.fws.gov/sites/default/files/2021-07/Striped%20bass%20Ryan%20Hagerty.jpg>

(right) Kanpachi (greater amberjack) in net-pen aquaculture. Photograph by NOAA Fisheries. <https://media.fisheries.noaa.gov/styles/original/s3/dam-migration/750x500-kanpachi-open-ocean-net-pen-blue-ocean-mariculture.jpg?itok=3AGeN75m>

Invertebrate-Specific Biosecurity:

(left) Blue mussel larvae. Photograph by Northeast Fisheries Science Center, NOAA Fisheries. https://www.fisheries.noaa.gov/s3/styles/media_250_x_250/s3/dam-migration/blue-mussel-larvae-2.jpg?itok=qj6-XSiL

(right) Rock scallop. Photograph by Pacific Shellfish Institute. <https://www.pacshell.org/images/scallop-beaker.jpg>

Seaweed/Macroalgae-Specific Biosecurity:

(left) Harvest of cultivated brown kelp in Maine. Photograph by NOAA Fisheries. <https://media.fisheries.noaa.gov/styles/original/s3/2021-07/seaweed-dropper-ropes-portland-maine.jpg?itok=gZhuUzFP>

(right) Seaweed salad. Photograph by K. Whiteford. <https://www.publicdomainpictures.net/pictures/210000/velka/seaweed-salad.jpg>

Examples of Region-Specific Biosecurity Issues:

(left) Oil sheen from a sunken supply ship. Photograph by U.S. Coast Guard. https://response.restoration.noaa.gov/sites/default/files/styles/gallery_active_image/public/images/%5Buid%5D/Coimbra2.jpg?itok=tqbwfDq4

(right) Satellite image of Hurricane Ian making landfall on Florida, September 2022. Photograph by National Centers for Environmental Information, NOAA. <https://www.ncei.noaa.gov/monitoring-content/billions/reports/20220928-20220930-tropical-cyclone/ian-satellite.jpg>

Plain Language Summary

Background

Aquaculture is the farming of water-based organisms, including shellfish, shrimp, salmon, and seaweed, among others. Currently, 21% of U.S. fisheries landings come from aquaculture, and the industry is expected to expand significantly in the coming decade. It will likely involve a range of participants, from small independent business owners to large, well established corporations.

As for any industry that relies on natural resources, the health and safety of both cultured and wild organisms are major concerns. This document summarizes guidance and best practices for disease management and biosecurity for marine aquaculture in the United States, including a report from a July 2022 workshop on best practices for disease management in marine aquaculture. This review relied upon peer-reviewed science, the observations and experience of aquaculture practitioners, and current regulations and policies, both domestic and international. Specifically, this document provides information supporting NOAA Fisheries' assessments of Aquaculture Opportunity Areas in the Gulf of Mexico and Southern California.

Key Takeaways

- Diseases pose as great a risk to aquatic organisms as they do to terrestrial organisms, ranging from loss of production to disease transfer between cultured and wild species.
- Biosecurity includes plans and actions to prevent the introduction and spread of diseases within a culture facility.
- A biosecurity plan for an aquaculture facility is a critical tool for preventing and managing disease. It requires good knowledge of the cultured organisms and the facility's operations to accurately identify hazards and actions to prevent and mitigate those hazards.
- There are common features for disease management and biosecurity for shellfish, finfish, and seaweed/macroalgae. These include appropriate stock selection, incoming water quality and security, quarantine, disinfection and decontamination, health and pathogen surveillance, and environmental monitoring.
- Each aquaculture sector (shellfish, finfish, and seaweed/macroalgae) has biosecurity needs specific to the type of cultured organism.
- The Aquaculture Opportunity Areas of the Gulf of Mexico and Southern California have region-specific issues that can affect biosecurity, including hurricanes, petroleum pollution, harmful algal blooms, wildfires, and pesticides.

Links used in this section:

- Aquaculture Opportunity Areas: <https://www.fisheries.noaa.gov/national/aquaculture/aquaculture-opportunity-areas>
- Risk to aquatic organisms: <https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/aquaculture/aquatic-animal-diseases/index>
- Biosecurity: <https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/avian/defend-the-flock-program/df-biosecurity>

Executive Summary

Marine aquaculture in the United States primarily produces bivalves (oysters, clams, mussels), crustaceans (shrimp), and finfish (salmon), and currently accounts for 21% of domestic fisheries landings. In 2020, the Executive Order on Promoting American Seafood Competitiveness and Economic Growth (E.O. 13921) was established. Among its objectives were to improve U.S. food security, facilitate permitting of aquaculture facilities, and provide environmentally safe and sustainable seafood. Federal support for offshore aquaculture, including ongoing efforts by NOAA to identify Aquaculture Opportunity Areas (AOAs), is likely to result in significant expansion of this industry in the coming decade. This expansion is expected to include marine finfish species other than salmon, and cultivation of seaweed/macroalgae. It is likely to involve a range of entrepreneurs, from small independent operators through larger established corporations.

Organism health, disease management, and biosecurity are major concerns for an industry that relies on natural resources. This document presents guidance for conducting marine aquaculture that protects the health of cultured organisms and the natural resources where an aquaculture farm is located. This review is based on peer-reviewed science, aquaculture practitioner observations, and existing regulations or policies within the United States or from nations with mature aquaculture industries. Furthermore, this review can inform and augment any national plan or policy governing marine aquaculture in federally managed waters.

This document is intended to be a readable reference; considerable effort has been made to keep the text succinct, accurate, and free of highly technical terminology. It begins with a short introduction to biosecurity, followed by disease management and biosecurity across the three aquaculture sectors (finfish, shellfish, and seaweed/macroalgae). The next sections present topics specific to each of these sectors. The next section presents examples of region-specific biosecurity threats for the Gulf of Mexico and Southern California. The final section is a summary of a workshop on marine aquaculture best practices that included participants from the aquaculture industry, disease researchers and diagnosticians, and government agencies.

Although technologies to monitor health and disease are constantly emerging, good husbandry and awareness of vulnerabilities to disease threats are at the heart of maintaining biosecurity. The guidance and practices described in this document consistently reach back to these fundamentals.

Acknowledgments

The authors are indebted to Roy Yanong (Professor and Extension Veterinarian, University of Florida), David Russell (Fish Pathologist, Maine Department of Inland Fisheries and Wildlife), Ryan Crim (Hatchery Director, Puget Sound Restoration Fund), and Janet Whaley (Lead Veterinary Medical Officer, NOAA Fisheries) for their reviews of this document. Kathleen Hartman (Senior Veterinarian for Aquaculture Health, USDA/Animal and Plant Health Inspection Service) reviewed the section on pathogen reporting. Their comments, suggestions, and edits substantially improved the quality of this document.

Glossary

Active surveillance: Collection, analysis, and interpretation of health and disease information using targeted methods and designs for a specific disease or pathogen.

Aquaculture: The breeding, rearing, and harvesting of fish, shellfish, algae, and other organisms in all types of water environments to produce food and other products, enhance wild stocks, restore declining wild populations or species, or recover wild threatened/endangered species.

Aquaculture sector: The portion of the aquaculture industry involved in rearing shellfish, finfish, or seaweed/macroalgae.

Biofilm: A collection of microorganisms that adhere to each other and to a surface, forming a living layer of cells. Biofilms can attach to both inanimate (e.g., rocks) and living surfaces (e.g., skin).

Biosecurity: The practices and measures taken to prevent the introduction or spread of harmful organisms onto, within, or from an aquaculture facility or system.

Carrier state: Condition when an organism is infected but not manifesting signs of infection or disease.

Chorion: Outermost membrane or envelope of an egg or embryo.

Culling: Population reduction via selective slaughter or depopulation. In the context of this document, it refers to killing organisms, either for consumption or non-consumption, to prevent the spread of disease.

Decontamination: Reduction of a contaminant to a level assumed to be reasonably free of transmission risk. Disinfection and sterilization are forms of decontamination.

Disease: A disorder of structure or function, especially one that produces specific signs or symptoms that are not simply a direct result of physical injury.

Disinfection: Elimination of most, but not necessarily all, infectious agents.

Early detection system: A system for ensuring the rapid recognition of clinical signs in an animal or population that are consistent with disease, specifically infectious diseases.

Endemic: Disease regularly found among particular organisms within a given population/area.

Epibiont: An organism that resides on the surface of another organism.

Exclusive Economic Zone (EEZ): The zone where the United States and other coastal nations have jurisdiction over natural resources. It extends no more than 200 nautical miles (nmi) from the territorial sea baseline and is adjacent to the 12-nmi territorial sea of the United States. The U.S. EEZ includes the Commonwealth of Puerto Rico, Guam, American Samoa, the U.S. Virgin Islands, the Commonwealth of the Northern Mariana Islands, and any other territory or possession over which the United States exercises sovereignty.

Fallowing: Placing a production site and equipment into disuse for a period of time to reduce or eliminate associated pathogens.

Farm-raised: Aquatic animals reared in controlled environments with intentional interventions to enhance animal production through feeding, husbandry, and protection from predators, with an implied ownership throughout the rearing period. Farm-raised animals may include animals reared for the purposes of enhancing wild stocks, restoring declining wild species or populations, or recovering wild threatened and endangered species. Those animals are privately owned until purchased and legally released by public or private entities.

Finfish: A bony fish, such as a salmon, or a cartilaginous fish, such as a shark, especially in contrast to a shellfish or other aquatic animal. In the context of this document, it will likely refer to animals reared in net pens in the marine environment.

Fomites: Inanimate objects that can transport an infectious agent. Examples include utensils, clothing, and airborne water droplets.

Grow-out: Phase in aquaculture in which juvenile animals are transferred to a location where they are held and fed until they attain the desired size for harvest.

Immersion calendar: A planning timeline for transferring shellfish to different water locations based on anticipated environmental conditions (including water temperature) to minimize mortality and disease exposure.

Integrated multitrophic aquaculture: A method in which two or more species representing different trophic levels are co-cultured to improve efficiency and reduce waste compared to single-species culture.

Invasive species: Living organism that is not indigenous or native to a geographic region and that is harmful economically, environmentally, or to the health and wellbeing of indigenous or native organisms.

Laboratory: A scientific facility engaged in conducting testing for the purpose of aquatic animal health inspection and diagnostics in support of aquatic animal health and aquaculture commerce.

Land-based: Occurring on land.

Mariculture: The subset of **aquaculture** that is **marine aquaculture**.

Marine aquaculture: Aquaculture where any portion of the production cycle is conducted in seawater.

Net pen: An aquaculture production system that confines aquatic animals to a specific location, typically in open-water settings. Synonymous with **sea cage**.

Non-indigenous species: Living organism that is not native to a geographic region.

Offshore: Rearing of aquatic organisms in controlled environments in federally managed areas of the ocean. Federally managed areas begin where state jurisdiction ends and extend 200 miles offshore, to the outer limit of the U.S. **EEZ**.

Passive surveillance: Collection, analysis, and interpretation of health and disease information using opportunistic, ancillary, or anecdotal methods.

Pathogen: An infectious organism that causes disease.

Pathogen of concern: Any pathogen that causes significant impact to aquaculture, aquatic animal production, and/or trade/movement. Includes, but is not limited to, pathogens listed by the WOAHO/OIE, USDA, and emerging pathogens.

Production calendar: Planning tool used to establish timepoints for husbandry actions from nursery through grow-out for the production cycle. Examples of information used for production calendars include organismal physiology, environmental conditions, and farm capacities.

Propagule: A part of a plant or alga that is used for vegetative reproduction.

Sea cage: An aquaculture production system that confines aquatic animals to a specific location, typically in open-water settings. Synonymous with **net pen**.

Seaweed/Macroalgae: Visible plantlike aquatic organisms containing photosynthetic pigments (e.g., chlorophyll) and typically attached to substrates such as rocks. There are three main categories: brown algae (Phaeophyta), red algae (Rhodophyta), and green algae (Chlorophyta).

Sentinel surveillance: Collection, analysis, and interpretation of complete and detailed health and disease information for a selected subset of the population that is assumed to be representative of the population.

Shellfish: Animals that dwell in water and have a shell, shells, or an exoskeleton. Examples include mollusks and crustaceans. In the context of this document, it refers to animals reared in marine or estuarine environments.

Specific pathogen-free (SPF): Animals that are guaranteed free of a particular pathogen(s).

Sterilization: Removal of all living microorganisms.

Quantitative trait loci (QTL): A locus that correlates with variation of a quantitative trait in the phenotype of a population of organisms. QTLs are mapped by identifying which molecular markers correlate with an observed trait.

Wild: Living as a free-ranging entity; not in captivity.

Abbreviations

AFS FHS	American Fisheries Society Fish Health Section
AOA	Aquaculture Opportunity Area
APHIS	Animal and Plant Health Inspection Service
BMP	best management practice
CAAP	concentrated aquatic animal production
CAHPS	Comprehensive Aquaculture Health Program Standards
CFIA	Canadian Food Inspection Agency
CONOPS	concept of operations
EPA	U.S. Environmental Protection Agency
ERMA	Environmental Response Management Application
FAO	Food and Agriculture Organization of the United Nations
IHHNV	infectious hypodermal and hematopoietic necrosis virus
IMTA	integrated multitrophic aquaculture
INAD	investigational new animal drug
ISAV	infectious salmon anemia virus
ISSC	Interstate Shellfish Sanitation Conference
NPDES	National Pollutant Discharge Elimination System
OIE	Office International des Epizooties, former name for the World Organisation for Animal Health
OSHA	Occupational Safety and Health Administration
OsHV-1	ostreid herpesvirus 1
OSPR	Office of Spill Prevention and Response
PEIS	programmatic environmental impact statement
QAAD	quarterly aquatic animal disease report
QTL	quantitative trait loci
RAS	recirculating aquaculture system
RSSBP	Regional Shellfish Seed Biosecurity Program
SPF	specific pathogen-free
TSV	Taura syndrome virus
USDA	U.S. Department of Agriculture
UV	ultraviolet
WAHIS	World Animal Health Information System
WOAH	World Organisation for Animal Health, formerly named Office International des Epizooties
WSSV	white spot syndrome virus



Introduction

Marine aquaculture, including offshore farms, is in early stages of development in the United States. New technologies allow equipment, such as submerged cages and multitrophic operations, to utilize federally managed waters within the U.S. Exclusive Economic Zone (EEZ). Managing diseases and pathogens that negatively affect cultured organisms is necessary for productive aquaculture and environmental protection. This document's purpose is to assemble guidance and best practices in disease management and biosecurity for marine aquaculture of finfish, shellfish, and seaweed/macroalgae. Recognizing that many marine aquaculture cycles extend beyond the grow-out period in marine waters, we have included practices applicable to freshwater, intertidal, and terrestrial parts of the production cycle. N.B.: Seafood safety is not within the scope of this document.

Disease Management and Biosecurity Across Sectors covers general and overarching biosecurity and disease management considerations for marine aquaculture, organized primarily by phases of the aquaculture production cycle. The following sections discuss practices and guidance specific to Finfish, Invertebrates, and Seaweed/Macroalgae, respectively. Examples of Region-Specific Biosecurity Issues addresses issues specific to the Gulf of Mexico and Southern California, where NOAA is making efforts to identify Aquaculture Opportunity Areas (AOAs). The sixth and final section is a summary of a workshop focused on the principal disease and biosecurity problems and responses by active marine aquaculturists of finfish, shellfish, and seaweed/macroalgae in the United States. The information provided by workshop participants strengthened this review with current and relevant knowledge.

Generalized Invasion Curve for Biosecurity

The emergence of a pathogen or invasive species typically has similarities in expansion that follow a logistic model, becoming limited by the number of susceptible individuals or by carrying capacity, respectively. The ability to manage the pathogen or invasive species is inversely related to that generalized curve (Figure 1). The most effective management tactic is to prevent introduction. Eradication of pathogens is still feasible during early stages and often involves culling hosts in conjunction with vaccination, if available. As the pathogen or invasive species spreads, management necessarily shifts to containment and longer-term measures, such as intensive surveillance and treatment. As the effort to manage spreading disease increases, the financial cost also increases, and the most expensive outcome is loss of organisms to preharvest mortality (Figure 1).

To prepare for each region of the curve, an aquaculture operation needs to be able to identify: 1) sources of risk for disease, and 2) actions to prevent and mitigate disease occurrences. To adequately address these needs requires:

- Complete knowledge of operations.
- Full understanding of diseases and pathogens that may occur, including susceptibility factors, preventatives, and therapeutics.
- Ability to conduct biotic and abiotic monitoring to detect both known conditions and conditions not previously encountered.
- Appropriate knowledge to document and properly report situations, including those required by permit conditions or regulation.

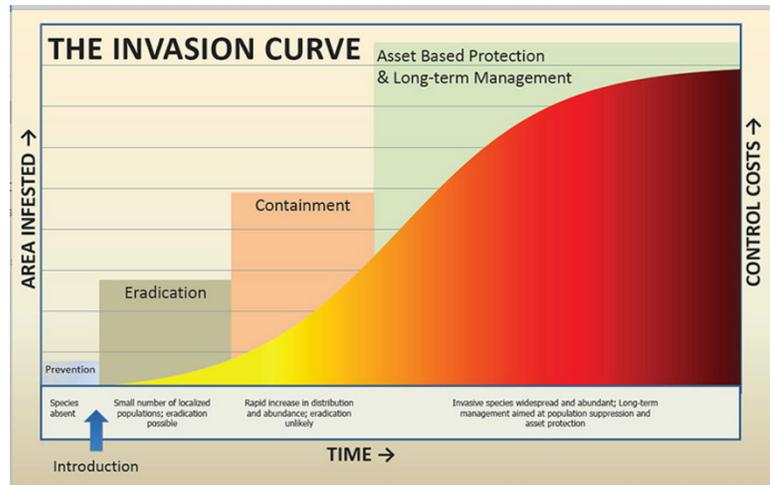


Figure 1. Generalized invasion curve for invasive species and pathogens. Source: U.S. Army Corps of Engineers. <https://www.usace.army.mil/Media/Images/igphoto/2000809105/>

A biosecurity plan can explicitly address these needs.

Biosecurity Plan

A biosecurity plan aims to reduce the risk of introducing, spreading, or releasing pathogens or disease as a result of aquaculture activity. The basic elements of a plan identify and characterize risks to organism health, describe actions to prevent and mitigate those risks, and define how risk management is communicated. These principles are embedded in the National Aquaculture Health Plan and Standards, 2021–23 (1), and the WOAHO/OIE’s Health Code Chapter on biosecurity for aquaculture establishments (2)—both excellent references for plan development. The most important feature of a biosecurity plan is that it is practical and effective. An early step in developing a biosecurity plan is knowing the routes of pathogen transmission (Figure 2).

Identify and assess hazards to organism health

This element requires knowledge of the pathogens and diseases known to affect the species under culture. Relevant information includes:

- Awareness of routes for introduction, spread, and release.
- Assessment of the likelihood of hazard of a pathogen or disease.
- Recognition and diagnosis of infection and disease signs.
- Identification of husbandry or management actions that affect risk.

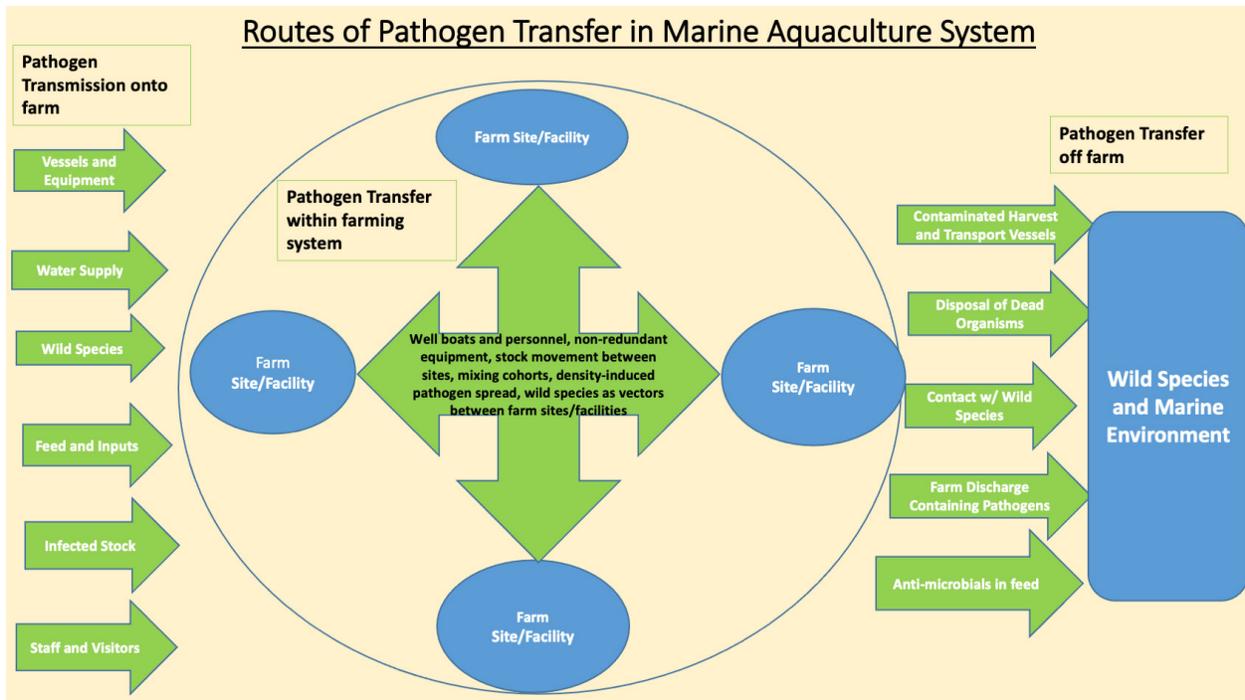


Figure 2. Simplified diagram of potential routes of pathogen transmission into, within, and from an aquaculture facility.

Describe actions to prevent and mitigate risks

This element requires knowledge of effective measures against the pathogens and diseases of the species under culture. Relevant information includes:

- Mitigation procedures for known routes of pathogen entry and spread.
- Role of infrastructure and engineering features (e.g., water supply, net-pen materials).
- Use of chemicals, prophylactics, and therapeutics.
- Procedures for documenting animal movements and husbandry activities to provide a log of operations that can be used for decision-making.
- Health monitoring plan and health monitoring records by farm personnel and by a qualified aquatic organism health professional, such as a veterinarian.

Define how risk management is communicated

This element focuses on ensuring that pathogen/disease risks and the preventative/mitigating actions are effectively communicated to personnel and visitors at the facility. Relevant aspects include:

- **Standard operating procedures (SOPs):** SOPs are site-specific, detailed, and practical, and they rely on research, experience, and industry standards. They are protocols for safe day-to-day activities and for actions to take in response to a pathogen- or disease-related event, including emergency measures (e.g., culling and quarantine).

- **Personnel training:** Initial and regular refresher training equips personnel with sufficient understanding of biosecurity principles to implement SOPs and to make responsible decisions in the midst of disease events.

Once a biosecurity plan is formulated and approved for implementation at a facility, copies of the plan need to be readily accessible on-site. Regular review of the plan and SOPs for updating due to new information, changes within the facility, or lessons learned from incidents, can keep them relevant and effective.

Templates and associated guidance for aquaculture biosecurity plans are available from several sources. In the United States, guidance including a biosecurity plan template (3) and information specific for recirculating aquaculture systems (4, 5) and freshwater ponds (6) are available. There are also excellent informational webinars about biosecurity that are publicly available.¹ The Commonwealth of Australia published useful guidelines for a generic template (7) and for a nonsalmonid finfish template (8). More recently, the government of New South Wales provided a broader template and guidelines for their aquaculture operators (9). Finally, the UK's Centre for Environment, Fisheries, & Aquatic Science published templates and associated guidance for marine finfish and shellfish aquaculture (10, 11).

¹<https://www.ncrac.org/video/what-you-need-know-about-biosecurity> and <https://www.ncrac.org/video/what-you-need-know-about-biosecurity-part-ii>



Disease Management and Biosecurity Across Sectors

Broodstock, Nursery, and Hatchery Biosecurity

Stock selection to reduce disease

Collecting wild organisms for broodstock or nursery stock is common for marine aquaculture. While this can preserve indigenous genetic lines and diversity, wild sources may also bring endemic pathogens with them into the facility. Developing specific pathogen-free (SPF) stocks can alleviate importing specific diseases into a facility. Excellent examples include penaeid shrimp diseases, white spot syndrome virus (WSSV), Taura syndrome virus (TSV), and infectious hypodermal and hematopoietic necrosis virus (IHHNV). These viruses were detected in wild shrimp in the Americas before 2000, and use of wild sources caused severe farm losses (12). The creation and use of SPF shrimp stocks for culture emerged as one of the best strategies for reducing these pathogens in shrimp aquaculture. For finfish, most SPF stocks are developed for research purposes, such as zebrafish (13), but adoption of SPF protocols for commercial fish is increasing, especially for broodstock and eggs.

Development and use of specific disease-resistant stocks enjoys broader application and research efforts. Efforts can range from more classical breeding approaches to employing quantitative trait loci (QTL) analyses during selection, depending on how much is understood about the genetics of the disease (14). Selecting for specific disease resistance without negative impacts on desirable traits (e.g., growth rate) is challenging, and inbreeding is always a risk (15, 16). When an industry reaches a scale where depth of genomic knowledge exists and investments in developing specific disease-resistant stocks provide measurable benefits, tools for selection and mapping, such as marker-assisted selection and genomic selection, become feasible (17).

Influent water control and management

Breeding, larval rearing, and other early life stages for marine aquaculture typically occur at a land-based hatchery, where vulnerable immature animals are reared before transplanting into the marine environment for grow-out. Management of the influent water source to any shore-based hatchery facility is probably the most important aspect of this part of the culture, and well recognized in the early days of hatchery operations (18). If using freshwater, such as for salmonids, surface inlet water can be more hazardous

than groundwater due to potential contamination from terrestrial runoff or by pathogens already present (19–21). Methods to reduce biological hazards in incoming water include mechanical filtration, ultraviolet (UV) irradiation, ozonation, and electrolysis (19–22). Mechanical filtration is a common concept, and some methods, such as sand filters, can remove particulates down to 20 μm . It is also frequently coupled to other methods, such as UV irradiation, to improve the effectiveness of subsequent treatment. Electrolysis of sodium chloride in seawater to chlorine dioxide can effectively kill microorganisms, but risks chlorine toxicity to aquacultured organisms; it is a rare choice for treating influent water.

UV irradiation

Natural (sunlight) and artificial UV light can directly damage microorganisms, and disinfection of seawater has been available for many decades (23, 24). One of the important benefits of UV treatment is that it does not generate harmful residues (25). Factors that can reduce the effective dosage include turbidity (organic and inorganic particulates), color (dissolved minerals), and declining bulb irradiation with aging. Filtration to remove particulates and bulb maintenance make UV irradiation an effective disinfectant, whether alone or in combination with other treatments (24). Effectiveness will also require knowledge and proper application of a given target organism's "zap dose" ($\mu\text{Ws}/\text{cm}^2$)—the dose required to kill or inactivate it.

Ozone oxidation

Ozone treatment is a highly effective disinfection method through oxidative reactivity with many compounds, including cellular membranes. Ozone is highly reactive with bromine, forming products that can persist at the pH of seawater, providing longer-term disinfection capabilities (24, 26). The persistence of bromine oxidation products and even ozone itself can pose a hazard for aquaculture organisms (26), depending on the amount of bromine in the seawater and the level of ozone injected, so removal may be needed before exposing the aquacultured species to treated water (25). Ozone can also deplete or reduce bioavailability of important nutrients by oxidation (e.g., iodine to iodate).

The methods mentioned above are often not practical for large-scale flow-through systems, and can generate undesirable byproducts that require removal before exposure to aquatic organisms. Increasingly, research and industry practice suggest that recirculating aquaculture system (RAS) culture is a best practice for hatchery culture for several reasons. Physical properties, such as pH, water hardness, salinity, and suspended organics can be more easily managed in RAS than in a flow-through system. RAS does require replenishment of water loss due to evaporation, spillage, or removal of sludge-bound water from the system; however, the small amount of external water (usually <10% of total volume) required is easier to control for water-quality factors (27–32). Critical nitrogen regulation in an RAS is managed by primary mechanical solids separation and biofiltration via microorganisms that play a strong role in oxidizing ammonium and nitrite; the microbial community structure varies depending upon the stages of organism growth (33).

Quarantine

The ability to isolate organisms from the larger aquaculture population or facility is a critical capability in husbandry (34). Quarantine is appropriate when transferring organisms between facilities or when organisms are suspected to be diseased (2). Quarantine is also used to allow acclimation to changes in water chemistry, recovery from stress (e.g., transport), or for observation of specific individuals. To function effectively, quarantine facilities are discrete and closed from the external environment, and include systems to treat influent and effluent water (3, 35). In addition to physical isolation, the practice of sourcing from a single population and holding them as a closed group during quarantine (also called “all-in-all-out” stocking) ensures stock integrity through quarantine (3).

Effective quarantine plans include the identification of potential pathogens, a minimum quarantine period, appropriate types of observations and testing, disposal protocols for dead animals, and a description of operating standards (35). Operating standards describe personnel actions to maintain isolation (e.g., clothing, gear changes, or decontamination; use of footbaths), types of decontaminating solutions (e.g., 20-ppm povidone iodine), and decontamination procedures (e.g., equipment contact time with decontaminants). A well designed quarantine facility and plan are two of the best methods for preventing the introduction of pathogens and invasive species to a facility.

Disinfection and Decontamination

Disinfection, in combination with good handling practices, can be very effective at disrupting the transmission of pathogens within and between facilities. Anthropogenic actions are a common route of pathogen transmission, and fomites are typically the mechanism of transmission (2). Fomites include equipment, personnel gear, vehicles, and any materials that contact the cultured organisms. Assessment of likely points of fomite transmission can identify how to prioritize decontamination, depending on the intent: prevention of pathogen entry or egress from the facility, minimizing spread of pathogens within the facility, or eradication (36). Disinfection is usually a combination of mechanical (e.g., scrubbing, drying) and chemical methods to inactivate or kill pathogens. However, the biocidal nature of chemical agents can also pose risk to nontarget and cultured organisms as well as humans, so disinfection must be conducted carefully when balancing pathogen management, organism safety, and human safety.

A disinfection plan requires: assessment of transmission routes; knowledge of pathogens to be controlled; safety precautions for staff, organisms, and environment; and criteria for the appropriateness of the disinfection process (36). Routine disinfection to manage pathogen spread will be less intense than emergency disinfection for a disease outbreak, because an outbreak’s higher pathogen load increases the risk of spread (36), and common, general disinfection protocols may not adequately target all potential pathogens (e.g., mycobacteria or crustacean parasites). Characteristics of the fomites, such as porosity or material composition, are important considerations for effective disinfection and avoiding negative chemical interactions. Disinfectants range from physical approaches such as desiccation, heat treatment, and UV irradiation, to chemicals that change pH, denature proteins,

disrupt membranes, inactivate enzymatic activity, and oxidize molecules (34, 36–38). The selection of a disinfectant or a combination of disinfectants is determined by the pathogens, collateral toxicity to nontarget organisms, feasibility of application, and legal use (36).

While disinfection strives to remove most pathogens, decontamination may be more feasible for complex equipment, such as vehicles, boats, and actual farm infrastructure. For example, prevention of spreading invasive species among freshwater systems may only be feasible by implementing decontamination procedures due to the traffic volume of recreational and commercial vessels (39). Several detailed manuals on inspection standards and for cleaning and decontamination of vehicles, heavy equipment, and vessels against aquatic invasives provide excellent guidance for an aquaculture decontamination protocol (39, 40).

Fallowing is ceasing to use equipment and a location involved in production, and it is typically done at the end of a production cycle to help reduce pathogen loading. Fallowed equipment is cleaned, sometimes decontaminated, and dried to destroy pathogens and disrupt infection cycles (e.g., sea lice) before a new group of susceptible organisms are introduced (41). Fallowing can be mandated in disease-free zones or as a condition of permitting, but most good biosecurity plans include fallowing. Fallowing is also used in finfish aquaculture to allow the benthic zone associated with the facility to recover from organic loading, such as from unconsumed feed and fecal matter (42–44), although recovery can be slow (43). Depending on the characteristics of the pathogen(s) targeted by fallowing (e.g., environmental persistence, transmissibility, presence of wild hosts or reservoirs), modeling can be applied to estimate the best fallowing strategy (e.g., synchronous or asynchronous; 45).

Health Monitoring and Disease Surveillance

Monitoring and surveillance needs

Regular health observations, inspections, and testing for diseases are fundamental to quality aquaculture. Observations by on-site aquaculture staff are often the most important feature of health monitoring, because those individuals have the greatest familiarity with the behavior and appearance of their charges, such as swimming behavior and feeding responses. Augmentations to staff ability to observe cultured organisms include underwater cameras and routine water quality measurements (e.g., temperature, dissolved oxygen, ammonia). Onsite staff are responsible for removing and inspecting mortalities, and are the first line of detection of an emerging health issue. These regular observations are important to document, so recordkeeping should be as easy as possible for staff. With the array of electronic entry devices now available, developing a health observation database with an easy-to-use interface is a smart investment.

Regular inspections of aquacultured species by a trained or certified health professional, such as an aquatic animal health inspector or fish pathologist certified by the American Fisheries Society's Fish Health Section,² is a second layer of health monitoring. These AFS FHS certified professionals possess the skills, knowledge, and experience to perform health inspections and to conduct disease testing for infectious and non-infectious

²<https://units.fisheries.org/fhs/certification/>

diseases according to established protocols in the AFS FHS *Blue Book* (46) or in the World Organisation for Animal Health/OIE's online aquatic manual (47). Certified fish health pathologists can also provide recommendations for managing disease issues. However, only veterinarians are permitted to issue prescriptions for drugs (48) or biologics (49); this option requires a valid veterinarian–client–patient relationship.

In the United States, there are no required inspections of aquacultured species by a certified professional or veterinarian unless there is a suspected reportable disease present or if there is a need to declare freedom from a particular disease or pathogen (1). Otherwise, aquatic health inspections and pathogen testing are voluntary. USDA/APHIS offers several options to voluntarily establish and maintain the health status of an aquaculture site and its organisms (1):

1. Participation in the Comprehensive Aquaculture Health Program Standards (CAHPS).
2. Freedom from specific pathogens at a specific aquaculture operation.
3. Negative test status for specific pathogens at a specific aquaculture operation within a cohort population.

To date, guidelines and guidance documents for ascertaining and maintaining the health of aquacultured organisms in the U.S. Exclusive Economic Zone have not yet been established, although fundamental principles have been defined, such as testing for specific pathogens of concern prior to stocking and the use of only feeds free from pathogens of concern (1).

In Canada, the Canadian Food Inspection Agency (CFIA) conducts disease surveillance through partnerships with industry, regional governments, tribal entities, and researchers in British Columbia and Atlantic Canada (finfish and shellfish), as well as in Alberta, Ontario, and Quebec (finfish only).³ These inspections can take place unscheduled or unannounced, in response to producer requests, for emergency purposes, for disease survey purposes, or as a follow-up to prior inspections.⁴ CFIA has established declared areas for disease status in the Domestic Movement Control Program; declared areas are different for finfish and mollusks.⁵

Types of surveillance methods

Different surveillance methods have differing abilities to detect pathogens or disease, and the chosen method is based on the desired information or monitoring objectives. A recent review of existing aquatic surveillance programs and publications produced a list of factors and actions to guide construction of an active or targeted surveillance program (50).

³<https://inspection.canada.ca/animal-health/aquatic-animals/diseases/surveillance/eng/1322933174051/1322933270922>

⁴<https://inspection.canada.ca/about-cfia/transparency/regulatory-transparency-and-openness/rights-and-service/processor-s-guide/producer-s-guide/eng/1326317067766/1326317301980>

⁵<https://inspection.canada.ca/animal-health/aquatic-animals/diseases/finfish/maps/eng/1450301136052/1450301136830#po>

Passive surveillance uses existing resources for data gathering, and, because it is less resource-intensive, it is the most common type of disease surveillance. Disease or pathogen information is often collected as part of another purpose, such as routine health examination. Advantages of passive surveillance are coverage of a large part of the aquaculture sector and the ability to detect emerging diseases (34, 51). For example, visual inspection of stocks can identify moribund individuals, and even though the cause may not be known, follow-up with diagnostic testing may identify the disease. The disadvantage of passive surveillance is under-reporting and lack of data that are suitable for epidemiologic analysis (51).

Active (or targeted) surveillance employs structured information collection which ensures the targeted information (e.g., presence or absence of a specific pathogen or disease) and associated contextual data are collected in a consistent manner. This type of surveillance is used when verifying that a population is free of specific diseases (52), and that the generated data are appropriate for epidemiologic and statistical analyses (51, 52). Contextual data include environmental parameters (e.g., water temperature fluctuations, patterns of nearby nutrient inputs) and biological information (e.g., seasonal harmful algae events, changes in farm worker activities). The principal disadvantage of active surveillance is the narrow target of detection, such as a single pathogen or disease. However, combinations of passive and active surveillance can be powerful for monitoring aquacultured populations, and permits for transport of living organisms often rely on both methods. Furthermore, active surveillance data can help extension, education, and regulatory bodies develop epidemiological models for disease prediction (53).

Sentinel surveillance in aquaculture collects structured disease or pathogen data from selected producers or locations to complement existing active and passive surveillance (51, 52). In a sentinel system, a small number of producers or locations provide detailed data on a regular basis. Selection of reporting units is based on known health/exposure status or strategic geographic location (16). Data can be collected from production organisms or from susceptible organisms positioned at the designated location. Although sentinel surveillance does require additional resources to implement, it can be effective in detecting emerging or uncommon diseases (51, 52).

Organism Transport Between Facilities

Although release of pathogens from a facility and subsequent passive transport by water is often considered an important mechanism for dispersion, human actions that actively move organisms and associated pathogens are a major way to transport pathogens over considerable distances (54). Transport of organisms from nursery/hatchery to grow-out sites, between grow-out sites, and from grow-out site to harvest or processing site present opportunities for pathogen transfer; health assessment prior to movement can prevent pathogen movement. Quantitative assessment of the spread of infectious salmon anemia virus (ISAV) among Scottish farms showed that the movement of live fish and harvest visits were associated with increased risk of infection, not simply the movement of personnel or equipment in the absence of fish movement (55). In Ireland, network modeling identified movement of subclinically infected fish as the leading risk factor in the spread of cardiomyopathy syndrome (56). Evaluation of Chilean salmon aquaculture identified fish transport as the greatest risk factor for movement of ISAV (57). For shellfish, the movement

of live organisms is identified as a major source of pathogen dispersion (e.g., ostreid herpesvirus 1, OsHV-1), both internationally and domestically in the United States (58). Health assessment prior to movement is especially important for situations where quarantine cannot be imposed at the recipient site, such as grow-out locations (35). The health assessment includes inspection by a qualified health professional, review of morbidity and mortality records, and testing for any specific pathogens of concern (9, 122). At a minimum, these features should be part of the aquaculture operation's biosecurity plan, and are likely to be a part of jurisdictional authority requirements before movement is authorized.

In addition to assurance of organism health status before movement, other biosecurity measures apply to transport vessels, handling equipment, and personnel gear to reduce the hazard of spread through contaminated fomites. Equipment and transport container decontamination, cleanable personnel gear, and proper personnel training are essential for biosecure transport (2, 35, 39, 40).

Organism welfare is critical for reducing pathogen susceptibility throughout the life cycle. It is particularly important a) during transport against pathogens that may be endemic at the receiving location, and b) for minimizing transport-associated losses, especially when quarantine is not an option. Controlling fish density, maintaining water quality, and using temperatures conducive to organisms (but not to pathogens) can reduce or minimize stress and susceptibility to infection/infestation from endemic pathogens and parasites at destinations (16, 59–61).

Passive transport of non-indigenous species and pathogens by human-based actions is a factor both within and outside of the control of the producer. Ballast water and vessel biofouling are major transport vectors of non-indigenous species and pathogens, historically and currently (62, 63). While aquaculture producers can minimize some hazards of transfer, they have little or no control over other commercial or recreational craft that traverse their production sites. Exposure to waterborne pathogens introduced in an area (e.g., infected bait used in crab or lobster fisheries) can also be beyond control except by siting away from established fishing areas.

Environmental Monitoring and Pathogen Reporting for Health and Biosecurity

Marine aquaculture is constantly exposed to natural phenomena that can threaten organism health and biosecurity, including hypoxia, harmful algal blooms (HABs), hurricanes, oil/chemical spills, and heat waves. Monitoring to anticipate and mitigate these phenomena can range from local measurements (e.g., water temperature) to large-scale monitoring (e.g., satellite imaging). Several environmental warning systems for the United States are available through the internet for marine aquaculture producers:

- National Hurricane Center and Central Pacific Hurricane Center.⁶
- Annual joint forecast of the extent and severity of the hypoxic “dead zone” in the Gulf of Mexico.⁷

⁶<https://www.nhc.noaa.gov/>

⁷<https://www.noaa.gov/news-release/noaa-forecasts-summer-dead-zone-of-nearly-54k-square-miles-in-gulf-of-mexico>

- NOAA's regional harmful algal bloom forecasts.⁸
- NOAA's *Vibrio* predictive models for safe shellfish harvest.⁹
- U.S. Coast Guard's National Response Center¹⁰ for oil spills, chemical releases, and maritime incidents.
- NOAA's Climate Prediction Center for proximal (6–10-day) through to distant (3-month) projections for temperature, precipitation, and hazards based on climate modeling.¹¹
- European Space Agency's Aquaculture project for sea surface temperature, phytoplankton, and terrestrial outflow (under construction in 2022).¹²

Warning systems developed by other countries, such as Scotland's HABs report website,¹³ which provides both reported detections and forecasts (64), can be a model for useful products for the United States.

Pathogens already present in or introduced to the marine environment can present challenges for marine aquaculture operations and natural resources alike. As a member country of the WOAHO/OIE, the United States is obligated to report confirmed detections of WOAHO/OIE-listed pathogens. Currently, federally accredited veterinarians, USDA-approved laboratories to conduct specific testing for listed pathogens, and state and federal authorities are responsible for reporting suspect and presumptive positive cases to state animal health officials and the USDA/APHIS. However, transparency of disease and pathogen presence varies by state, and notification of detections to at-risk private and public aquaculture operations is challenging because of confidentiality concerns and inconsistent reporting.

Countries and regions with longer histories of aquaculture have publicly accessible information for high-priority pathogens such as sea lice.¹⁴ In conjunction with FAO and WOAHO/OIE, regional aquaculture organizations, such as the Network of Aquaculture Centres in Asia-Pacific, established the Quarterly Aquatic Animal Disease Report (QAAD) system in 1998, and that system continues today.¹⁵ Member countries of the WOAHO/OIE are obligated to submit information on detections of WOAHO/OIE-listed pathogens, and may voluntarily provide their self-determined status for pathogen freedom. Depending on the incident, reporting by the country's competent authority to the WOAHO/OIE occurs immediately or every six months using the WOAHO/OIE World Animal Health Information System (WAHIS) database.¹⁶ These and similar reporting databases are useful for identifying "hotspots" of specific diseases geographically and for trend analyses.

⁸ <https://coastalscience.noaa.gov/research/stressor-impacts-mitigation/hab-forecasts/>

⁹ <https://products.coastalscience.noaa.gov/vibrioforecast/>

¹⁰ <https://nrc.uscg.mil/>

¹¹ <https://www.cpc.ncep.noaa.gov/>

¹² https://www.esa.int/Applications/Observing_the_Earth/Envisat/New_ESA_project_supports_aquaculture

¹³ <https://www.habreports.org/>

¹⁴ <https://www.gov.scot/publications/fish-health-inspectorate-sea-lice-information/>

¹⁵ <https://enaca.org/?id=8>

¹⁶ <https://wahis.woah.org/#/home>



Finfish-Specific Biosecurity

Production Cycle

Marine finfish aquaculture in the United States currently includes salmonids and *Seriola* spp. (65–67). While production cycles differ for anadromous species and animals that live entirely in seawater, there is significant overlap in biosecurity and disease prevention protocols (21, 28, 31, 68, 69) that apply to marine finfish aquaculture. Additionally, some disease prevention and biosecurity methods for finfish are not available for macroalgae or shellfish culture, such as vaccination (34, 53). The production cycle begins with spawning in a controlled environment, usually a shore-based hatchery, where animals are hatched and reared until they are moved to the marine environment for grow-out until harvest. During these stages, there are multiple points of concern for biosecurity and disease control.

Broodstock, Nursery, and Hatchery

Most finfish broodstock and their eggs are derived from wild-captured animals or, in the case of anadromous species, adults returning from the ocean. Although broodstock can be quarantined, their value may be sufficiently high to retain even symptomatic individuals for spawning. For high-value broodstock such as Atlantic cod, sablefish, *Seriola* spp., cobia, and tuna, surrogate or progeny testing in lieu of lethal sampling of adults can be used to establish pathogen freedom for populations that are held for multiple spawning cycles. Surrogates can include smaller or younger fish of the same species co-reared with broodstock or the direct untreated effluent from broodstock. On the other hand, asymptomatic carriers of pathogens, especially viruses, may persist well in quarantine and never break with disease. In all cases, the potential for vertical transmission is substantial. Testing adults for pathogens is usually limited to nonlethal methods if spawning is not terminal. Methods such as testing tank water, mucus, or small biopsy samples (e.g., gill clips) are useful for adults held for more than one spawning cycle (e.g., 70). For fish species that undergo a single terminal spawn, the ovarian fluid is an excellent source for pathogen testing (e.g., 71, 72), although most culturists will also test another target tissue such as spleen and posterior kidney for systemic infections and heart, gill, or brain for pathogens that target those organs.

Culling or segregation of eggs based on broodstock pathogen testing can be effective in reducing vertical transmission (73, 74), and remains a best practice for pathogen control even though reducing vertical transmission could make horizontal transmission relatively more important

and drive a more virulent pathogen (75). In the case of high-value broodstock that are infected with bacteria or where bacterial infection status is unknown but suspected, broodstock have been injected with antibiotics with effectiveness against the specific bacterium (76–78); however, antibiotic use is increasingly discouraged due to risks of resistance development.

Egg surface disinfection after fertilization is commonly performed using chemicals such as formalin, povidone-iodine, hydrogen peroxide, or peracetic acid/hydrogen peroxide (79, 80). Disinfection by ozonation is another successful method that works well with pelagic broadcast spawners (81, 82). One caveat about egg disinfection is that it may not be effective against pathogens that are vertically transmitted intraovum due to protection by the chorion.

Management of incoming water is crucial for nurseries and hatcheries, particularly for anadromous species that can move upstream of a freshwater intake and release pathogens into a stream or river (21). Surface freshwater water (instead of groundwater) and seawater (for smolt acclimation) contain an abundance of microorganisms which can be reduced through disinfection and water reuse (21).

Pathogen transmission relies on having susceptible individuals to receive the pathogen. In nurseries or hatcheries, the use of multiyear cohorts can create a situation where susceptible individuals enter the facility multiple times, potentially initiating an epizootic among the susceptible cohorts. Single cohort stocking (also known as the “all-in-all-out” approach) can help minimize pathogen transmission at both nurseries/hatcheries and grow-out facilities.

Disinfection, Decontamination, and Cleaning

Marine aquaculture of finfish is most likely to involve net pens. Traditional pen materials such as polymers have high susceptibility to biofouling, which not only reduces water flow into the pen (if not isolated from surrounding water), but can add weight and hydrodynamic drag to the entire structure (83). Although materials such as copper alloy mesh can help to reduce biofouling, often the most efficient system involves physical removal by divers. Similar to personnel gear above water, scuba, wetsuits/drysuits, and defouling equipment need decontamination when moving between farm sites to prevent transfer of pathogens and potentially invasive organisms. Typical treatments include freshwater iodophor immersion (>100 mg/L free iodine) or freshwater immersion heat treatment to $\geq 60^{\circ}\text{C}$ for ≥ 2 minutes (84).

Because fish transfers involving well boats are a point of considerable risk for pathogen transmission and dispersal, cleaning and disinfection of boats and associated equipment are essential for biosecurity. Risk management options based on differing operating scenarios (e.g., movement within vs. between management zones) can define the degree of decontamination required (84).

Dead and moribund fish need to be removed as soon as feasible to reduce spread of any infectious cause of death and to prevent water quality decline due to decaying carcasses (4). Increases in daily mortality rates above background can be detected through recordkeeping, and case definition criteria need to be established for known diseases. Disposal of dead fish

is subject to regulation by regional authorities, although U.S. Clean Water Act compliance developed by the EPA for concentrated aquatic animal production (CAAP) provides excellent guidance for handling carcasses from flow-through, recirculating, and net-pen facilities (85).

Following periods are relatively well established for salmon reared in temperate zones, with 4 weeks established as a minimum time, and 3–6 months if a notifiable disease has occurred and the equipment is to be fallowed in situ (84).

Grow-Out

Animal monitoring

Traditional inspections and audits for pathogens or disease signs typically involve visual inspections by a qualified fish health professional. However, strict thresholds for certain parasites such as sea lice have stimulated a variety of technologies for monitoring, prevention, or treatment. Digital imaging, including underwater hyperspectral imaging, is being adapted for counting the number of lice per fish, which is the regulatory metric in Norway and Scotland (86, 87). This technology, combined with artificial intelligence (AI), has potential to replace direct inspection by humans with remote monitoring (e.g., Imenco's high-resolution fish health camera¹⁷). Remote camera technology also has the potential for improving visual observations by health professionals and optimizing feeding strategies based on animal behavior.

Environmental monitoring

While natural phenomena can affect aquaculture, finfish farm activities can affect the environment, which is the driver for required environmental monitoring around finfish farms by the EPA.¹⁸ Monitoring prescribed in a National Pollutant Discharge Elimination System (NPDES) permit is farm-specific, and in some cases the authority is delegated to the relevant state authority for water quality. Monitoring farm-associated benthos is a high priority for reducing adverse impacts (e.g., oxygen stress) and potential for harboring pathogens that can survive in organic matter-rich environments. Although there are frameworks available (e.g., 88 for benthic monitoring), a suite of relevant abiotic factors can be monitored in adjacent pelagic waters as well, such as dissolved inorganic nutrients (reviewed in 89). Aside from monitoring required by NPDES permitting, there is currently no mandatory environmental monitoring for farms located in federally managed waters.

¹⁷https://imenco.no/product/camera-system-insight?gclid=CjwKCAiAuaKfBhBtEiwAht6H764YgmbXL_HSaeQ4YUQ-qh3g2zLafS8NbtTGxu9Kf8Zz-wG1AV2tERoCxtYQAvD_BwE

¹⁸<https://www.epa.gov/npdes/aquaculture-npdes-permitting>

Prevention or treatment

Methods to prevent ectoparasite infestations are most advanced for sea lice. Some infrastructure devices that exploit parasite preference for surface waters include skirts positioned around the pen or cage at the surface; perimeter bubble curtains; net roof barriers that keep fish deeper with a protected vertical tube or “snorkel” that allows fish to swim to the surface for swim bladder replenishment; and sea lice traps positioned around the exterior of the pen or cage (90, 91). Treatments for sea lice during grow-out include feeds containing antiparasitic drugs such as avermectin (e.g., SLICE), external chemicals (hydrogen peroxide), mechanical flushing with water jets, and exposure to sudden water temperature or salinity changes (90, 91). Biological control of ectoparasites is an emerging area of nonchemical treatments. Incorporation of cleaner fish, such as lumpfish or several species of wrasse, into the grow-out culture is being trialed for effectiveness (91, 92). Cleaner fish pose a lower environmental hazard than chemical treatment and a lower trauma hazard to the cultured species than flushing, thermal, or salinity treatments, but the cocultivation of a second species in the grow-out facility can be an added biosecurity hazard (reviewed in 92). These hazards include the introduction of pathogens associated with cleaner fish into the culture (92), or cleaner fish serving as a reservoir for pathogens of the primary cultured fish (93).

Biofouling of net material (typically composed of a synthetic polymer such as nylon) poses three different kinds of risks for biosecurity: serving as a substrate for pathogen or non-indigenous species attachment, impede of the flow of oxygenated water, and increased hydrodynamic drag on the net or pen structure, which reduces water flow and increases mechanical stresses. Copper alloy mesh or netting have lower drag coefficients than traditional materials (94), and field testing indicates that production output measures equal or exceed those of systems using traditional materials (95, 96).

Feeds

Healthy organisms rely on high-quality feeds that adequately fulfill nutritional requirements. Formulations from feed manufacturers contain ingredients reviewed and approved through a collaboration of FDA and the Association of American Feed Control Officials.¹⁹ Commercial feeds have become much safer for finfish since implementation of heat-treatment or pasteurization to kill pathogens in the raw ingredients, which often include by-products of fish processing plants. However, there are certain pathogens, such as *Salmonella*, that can be heat-resistant or survive air-drying or freezing (97). Although there is little evidence that *Salmonella* in feed has negative effects on finfish, contaminated feed can pose an infection risk to other wildlife (e.g., marine mammals) who have access to the feed (97). Secure storage of feed against pests, such as rodents and wild birds, is also important as these animals are another source of *Salmonella* (98).

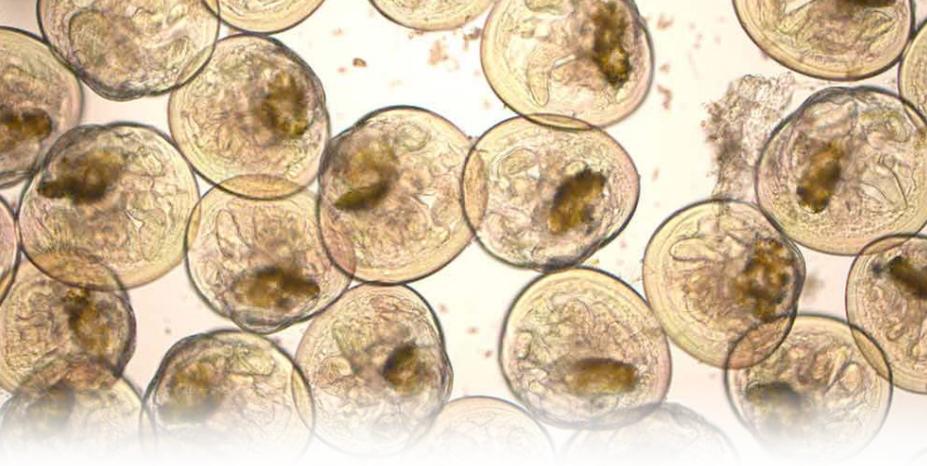
¹⁹<https://www.aafco.org/>

Food that has been frozen but not pasteurized, such as bait fish, can introduce pathogens, as not all are killed by freezing. Imported bait for feeding ranches tuna is suspected of introducing pilchard herpes virus to populations of native pilchard in Australia, resulting in epizootic mortalities, and that virus is now endemic in the area (99). For wild-captured broodstock (e.g., red drum), it may be necessary to feed fresh or fresh-frozen fish or invertebrates to assist transition to commercial diets or to fulfill nutritional requirements. The noncommercial diet will not be subject to feed quality standards and inspections (e.g., 100) and could be a route of pathogen entry. Testing expense for fresh or fresh-frozen feeds may not be cost-effective if the hazard of transmission is low, and using healthy-appearing prey is preferred.

Feed storage (which can be considerable in volume for large operations) needs to be secure from pests and stored to preserve feed integrity (e.g., minimize fatty acid oxidation) and to avoid feed-related disease conditions, such as liver tumors caused by fungal aflatoxins (101).

Transport and Harvest

One of the greatest concerns regarding transport is the use of well boats, or boats with the capacity to transport fish and equipment. The cost of acquiring and maintaining transport vessels dictates that they often visit multiple farms in a day (21), presenting opportunities for pathogen transfer between sites or for disseminating pathogens into the environment. Vessel disinfection between farms or designated health management zones for fish transport is a primary method for reducing pathogen transfer (102). Older vessel designs may be difficult to disinfect, whereas newer designs are often equipped for disinfection (21). Flow-through wellboats are not acceptable. Route planning ideally prioritizes farms with higher health conditions before traveling to farms with lower health conditions, or farms with younger fish before those with older fish (102). Ballast water is another route of pathogen transport, and Norwegian salmon operation guidelines recommend that ballast water not be taken in or discharged by watercraft within 5 km of any marine aquaculture facility (21). These factors can be incorporated into a transport or harvest plan, or a “concept of operations” (CONOPS) that maps the sequence of routes and includes fish spill response actions (21, 102).



Invertebrate-Specific Biosecurity

Invertebrate aquaculture in the United States involves primarily bivalve mollusks and crustaceans. By volume, oysters, clams, and mussels are the most-produced invertebrates in the United States, with a smaller number of abalone, scallop, and shrimp produced (103).

The use of microalgal pastes is becoming increasingly common for larval and early juvenile phases, but excepting shrimp, marine invertebrates cultured in the United States typically do not receive formulated feed during grow-out. Bivalves filter-feed from their surrounding environment while gastropods graze on algae; both feeding modes are primary mechanisms for pathogen acquisition (104). Principal factors in invertebrate disease management include: husbandry for optimal animal welfare; appropriate management of culture density; movement and transplantation of animals; health surveillance; and testing and reporting standards (105).

Production Cycle

There are three stages of the invertebrate production cycle with differing biosecurity needs: hatchery, nursery, and grow-out (105). Hatchery phases typically occur on shore in facilities that intake and treat seawater for controlled spawning and rearing of larval organisms. Nursery operations can continue on land or involve outplanting into seawater using containers or gear to provide physical protection to vulnerable juveniles. The grow-out phase occurs in marine or estuarine environments (including ponds provided with seawater) where the animals extract nutrients and organic matter from the ambient environment (bivalves, most crustaceans) or are fed a commercial feed (shrimp). Throughout the production cycle, physical separation of different life stages is an important prevention of disease spread due to different disease susceptibilities of life stages and the possibility of carrier states.

Seed Production and Hatchery

Broodstock and seed

Broodstock and seed procurement are often performed by entities distinct from production entities, or are collected from the wild. Moving broodstock and seed is an important mechanism of pathogen and disease transfer (106), and persistent harvest of wild broodstock can both pose a threat to wild stocks and be a constant source of pathogens (107).

Development of disease-resistant broodstock is an appealing objective, but this has been realized for only a few shrimp and oyster species (15, 108). Although co-selection of undesired traits and difficulty in selecting resistance to multiple pathogens are confounding issues, a careful breeding and challenge process has potential to select for wider robustness against infection (15). Another approach is development of specific pathogen-free (SPF) organisms (109). Although SPF populations are typically free of a single pathogen, the high level of maintenance biosecurity provides additional protection against a broader spectrum of diseases (109). Like disease-resistance breeding, SPF has been successfully used in shrimp aquaculture, though not in other types of invertebrate aquaculture (12, 109).

A recent initiative to create a Regional Shellfish Seed Biosecurity Program (RSSBP) addresses better seed source security and streamlines the movement of shellfish along the U.S. East Coast (106). The program is predicated on the very low risk of pathogen transfer presented by shellfish seed maintained on filtered water. It prescribes health examinations, management practices, and recordkeeping, including third-party audits, for a facility to declare disease freedom (106). Participation in the program is voluntary, and compliant hatcheries have two levels of organism movement, regional and coastwide, overcoming a myriad of jurisdictional boundaries (106).

Intake water quality management

Biosecure seed production and broodstock spawning occur in onshore hatcheries that take in marine or estuarine water for production tanks. Untreated water poses obvious risks of introducing pathogens, especially if conspecifics or closely related species of the cultured organism(s) live near the intake water source. In larval culture, best practices for treatment of intake water for flow-through systems include filtration (recommended pore size 1 μm ; 106), UV filtration, and ozonation (16, 24, 25). For broodstock and juvenile nursery culture in flow-through systems, filtration to 1 μm is not practical. Recirculating aquaculture system (RAS) hatcheries can provide secure water quality and reduce the volume of replacement water required (110), and these qualities make RAS production an effective choice for shellfish seed hatcheries (27, 29, 105, 111). However, depletion of calcium in recirculated water due to shell formation may require calcium supplementation (27).

For all hatchery situations, separation of broodstock, larval, and algal culture (for larval feeding) from untreated intake water is achieved through discrete plumbing and by distance to avoid splash contamination. Additionally, as a general rule, each piece of equipment has a specific operational area to avoid transfer of untreated water into the hatchery (106).

Nursery and transport

A nursery phase often follows the hatchery phase, allowing animals to acclimate to the natural ambient water before being moved to their grow-out location. The ambient sea or estuarine water is a potential source of pathogens (105, 106), and monitoring for pathogens of local concern is a good practice, especially when seasonal or water quality conditions indicate a higher possibility of disease occurrence (16). An active surveillance effort for known problematic pathogens will improve nursery phase biosecurity.

Transport of organisms from hatchery to nursery, or from nursery to grow-out, is best accomplished by minimizing exposure to uncontrolled environments during transport, such as a flow-through water system on transfer vessels. Decontamination of transfer vessel wells or transfer containers between batches is an obvious action. A transfer or CONOPS plan, which includes a spill response protocol, can help to avoid moving from sites with older organisms to younger organisms. When feasible, quarantine prior to moving to the grow-out location is the best way to avoid pathogen transfer (105), especially when the grow-out location already includes cultured animals. In many states (e.g., Washington), transfer permits must be issued by regulatory authorities. These permits may require a health exam by a certified shellfish pathologist and no detection of pathogens of concern.

Because human workers perform manipulations of stock and equipment, training and experience for everyone involved in the production cycle is critical. Training is best provided as a hands-on process, including every person from the highly skilled aquaculturist to the volunteer worker. Training is a part of the biosecurity plan for the facility.

Grow-Out and Harvest

The grow-out phase is the longest part of the production cycle; it can last several months, and over a year for many invertebrates. Animals are periodically sorted during the grow-out phase as they grow larger to adjust for animal density and to allow for maximum growth. This sizing and movement of animals is a source of stress for organisms and opens an opportunity for pathogen transport to the site by workers. Selecting and applying these management techniques in an integrative approach can be customized for specific sites using husbandry expertise and local knowledge (16, 53, 112, 113).

Unlike finfish, invertebrates lack an adaptive immune system (53), making vaccines ineffective. Therefore, prophylactic measures to reduce disease hazards are primarily population management actions, such as controlling stocking density and culling. A higher stocking density has been documented to increase the risk of disease transmission and decrease the growth rate (29, 53, 104, 111, 112, 114, 115). Generalized mortality increases with stocking density, and properly managed densities for each species can be an effective, low-cost tool to achieve biosecurity (16, 53, 112). If there are few data on recommended stocking densities for the species or location, empirical trials may be needed to determine the optimal stocking density for disease prevention (105). If a disease event occurs, reducing density by culling animals exhibiting signs of disease also removes active pathogen sources (53). A culling strategy is less effective for diseases where the clinical signs are not obvious until after an animal has died, such as oyster herpes virus (53). For diseases with few clinical signs or for smaller individuals that are difficult to monitor, a species-specific density to preempt disease maintenance in a population is a better approach (53). Finally, the elevation level of cultured animals is important in areas of significant tidal fluctuation (16, 113). For example, research suggests that a higher cultivation structure for adult oysters can reduce mortality by half in intertidal zones during periods of high risk for OsHV-1 (113).

Production and immersion calendars, which use an integrated approach to invertebrate farm planning, have been shown to be critical for invertebrate aquaculture to reduce the impact of diseases that predictably occur at certain times of the year (16). Production calendars consider the physiological development stages of animals and environmental conditions, such as temperature, that coincide with increased disease mortality, exemplified by OsHV-1 in Europe. Although the virus persists in a wide range of temperatures and is impossible to eliminate from the natural environment (53), immersion calendar planning can significantly reduce the mortality of animals (112). Production calendar planning in concert with reduced animal density and biosecure production practices can increase the numbers of animals that survive until a desired harvest date (112). Obviously, local environmental variations and local pathogens mean an immersion calendar will be specific to a farming location.

Invertebrate harvest conditions can be planned to avoid conditions that are unfavorable, such as temperature and tidal state. A good practice for harvest is to minimize differences between the immersion temperature at the grow-out location and the temperature during transport to reduce the flux-related stress response by the organisms (16).

Disinfection, decontamination, and cleaning

Depending on the culture method and species under cultivation, different cleaning and decontamination strategies are available for invertebrate aquaculture. For example, bivalves may be cleaned of epibionts and then immersed in a 60-ppm chlorine bath for 1 hr prior to introduction into a hatchery system. Handling one species at a time and disinfecting equipment before and after use help to prevent cross-contamination. When culturing in baskets or in other enclosed structures, cleaning equipment, such as abrasive brushes and other equipment, may be necessary to remove biofouling (116). Biofouling on structures can exacerbate environmental stressors, such as reduced water exchange, and can retain pathogens or other undesired organisms such as invasive invertebrates and harmful algae (116).



Seaweed/Macroalgae-Specific Biosecurity

Disease management and biosecurity for seaweeds and macroalgae is rapidly expanding as this area of mariculture grows. Tropical seaweed culture of eucaumatoids has provided many lessons learned (117), resulting in useful and practical documents for farmers, such as *Standard Operating Procedure of Eucaumatoid Cultivation Using A Biosecurity-Based Approach* (118). In temperate zones, seaweed/macroalgae culture is considered for monoculture and for integrated multitrophic aquaculture (IMTA) systems (119), and biosecurity issues will differ based on application. A useful and practical document for temperate zone monoculture is *Best Practice Guidelines for Seaweed Cultivation and Analysis* (120).

Broodstock, Nursery, and Hatchery

Similar to finfish and shellfish, traceability of propagules and seedlings to sources with a history of low disease or infection rate is a primary concern. Although indigenous strains can be advantageous because they are adapted to the local environmental conditions, quarantine or isolation before outplanting is beneficial in screening for individuals in poor condition and avoiding introduction of a pre-existing infection or infestation to the farm site (118). Selection of stocks with natural resistance to disease is not well developed for seaweeds and macroalgae, and repeated vegetative propagation and potential reduction in genetic diversity (117) may be related to loss of disease resistance during cultivation (121, 122). Nonetheless, breeding and propagation technologies for seaweeds and macroalgae are improving (123), offering the potential for disease-resistant, locally adapted strains for aquaculture.

Handling and inspection of propagules and seedlings are important for maintaining quality prior to establishment on the farm. Minimizing air exposure of thalli reduces the risk of desiccation stress (124). Inspecting and discarding propagules that are discolored, damaged, or covered with biofilms or attached algae helps to ensure that good quality stocks are installed at the farm (124).

Hatcheries for kelp propagation have many of the same requirements that shellfish and finfish hatcheries need: a biosecure water supply; the ability to adjust water quality and condition; facilities and equipment that can be regularly cleaned and disinfected (120). However, kelp hatchery activities involve handling microscopic organisms and culturing in a nutrient medium, imposing additional levels of sterility. Supplies must be maintained as sterile as possible, including autoclaving; employing aseptic techniques is necessary to minimize contamination (120). If gametophyte culture is conducted, incubators with lights

providing appropriate wavelengths to support spore development or stimulate seeders are required (120). After seed deployment, shutting down the hatchery to sterilize equipment and containers can disrupt contamination carry-over (120).

Introduction of non-indigenous seaweed/macroalgae is a particular concern for aquaculture, given the disastrous establishments that have been documented (125). Some introductions have been deliberate; in other cases, they were unintentional as a byproduct of other aquaculture activities (e.g., imported shellfish; 126). The ability of seaweeds/macroalgae to significantly modify habitat and alter energy flow in food webs underscores the importance of not cultivating non-indigenous seaweed/macroalgae.

Disinfection, Decontamination, and Cleaning

The use of cleaned and disinfected equipment, including ropes, rope-tying platforms, and transfer containers, reduces pathogen and epiphyte transfers between crops and between farms (124, 127). Sun-drying the ropes is an inexpensive and cost-effective method of disinfection (127). In temperate zones, accumulation of epiphytes and biofilms is a seasonal process often managed by timing of harvest (119), and removal of biofouling from the initial propagules and seedlings has been effective in reducing disease for tropical species (124).

Health Monitoring and Disease Surveillance

Although labor-intensive, regular inspection for surface growth and removal of heavily affected plants is effective in reducing disease in tropical species (124). In temperate zones, biofouling has a strong seasonal component (e.g., bryozoan recruitment), so management is typically through controlling growing and harvest timing (119).

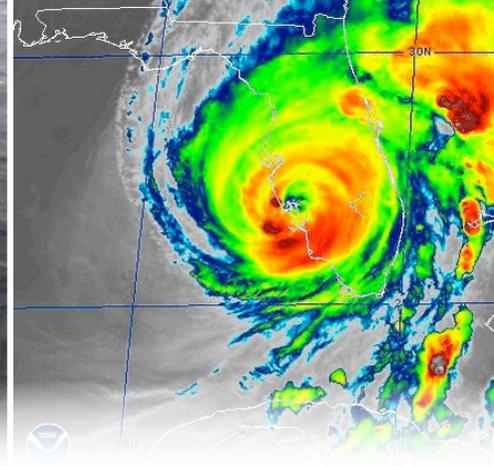
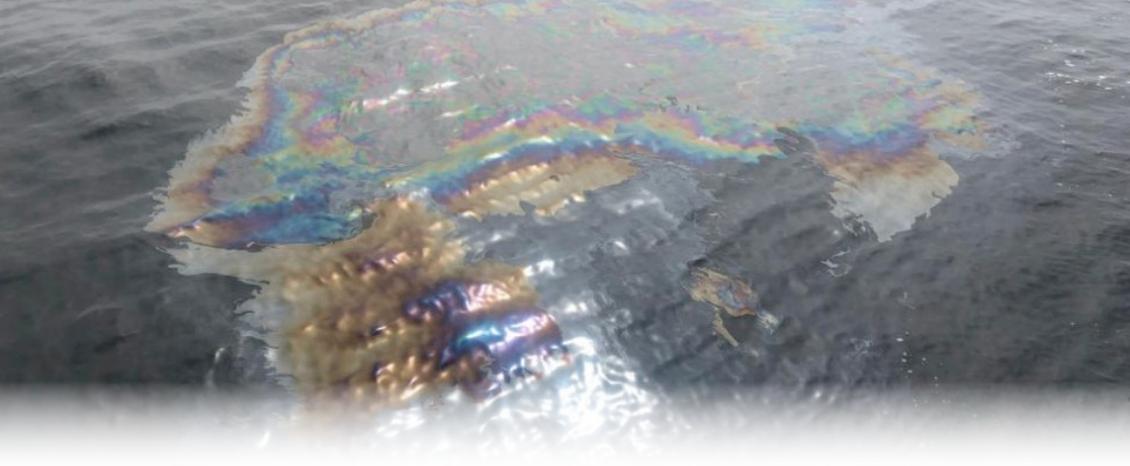
Transport, Grow-Out, and Harvest

Due to the relatively large area required by seaweed/macroalgal culture and the hydrodynamic changes caused by the growing crop, the grow-out site needs to provide stability in temperature and salinity while maintaining good water flow for nutrient replenishment and minimizing holobiont attachment (121).

Protecting propagules or seedlings against desiccation and mechanical damage during transport to grow-out locations is important for both tropical and temperate seaweeds/macroalgae (120, 121, 124). For eucaumatoid seaweed, acclimation of newly planted crop to below 50-cm water depth during the first 10 days can improve crop early survival (124).

If the crop is regularly inspected and culled of unhealthy individuals, waste material is best disposed of away from the farm (e.g., landfill) to reduce ambient bacterial load in sediments (128). Because seaweed/macroalgae serve as a substrate for other biological organisms, there is a hazard of co-transporting undesired organisms, even with healthy-looking crops.

After harvest, cleaning and fallowing or cleaning and disinfection (e.g., sun-drying) of equipment is used to break pathogen transmission between crop cycles (127).



Examples of Region-Specific Biosecurity Issues

In May 2020, Executive Order 13921, “Promoting American Seafood Competitiveness and Economic Growth,” mandated the development of an offshore marine aquaculture plan for the United States. In August 2020, NOAA identified two regions for further evaluation for Aquaculture Opportunity Areas (AOAs), the Gulf of Mexico and Southern California. The following sections discuss biosecurity topics specific to these two regions.

Gulf of Mexico Region

Hurricanes

Growers in the Southeastern region of the United States are familiar with the threat of destruction and loss due to hurricanes. The U.S. Department of Agriculture’s Southeast Climate Hub²⁰ developed a guide to assist pond-based aquaculture to prepare and recover from hurricanes, and many of the same principles can be applied to marine farms (129).

Protection against loss under the Federal Crop Insurance Corporation²¹ is not extended to aquaculture, requiring producers to obtain private insurance, if possible. Regardless of underwriter, documenting the farm inventory is necessary, and an emergency action plan is required by the Occupational Safety and Health Administration (OSHA) for farms with ten or more employees. A farm emergency plan can detail the facility response to a hurricane, suggest preparations for a worst-case scenario, and help ensure continuity of operations. For example, farms with submersible cage systems may be able to avoid much of the force of a hurricane by lowering cages to a depth of 50 ft (130).

Hurricane impacts on aquatic species include: high nutrient loading with subsequent hypoxia or anoxia; rapid salinity changes due to storm surges; sediment disruption creating turbidity and mechanical interference with respiration; degraded water quality due to suspension of anthropogenic contaminants in sediments or releases from overwhelmed industrial facilities (including wastewater treatment plants); release of urban, household, and agricultural debris and contaminants; and redistribution of wild stocks (131).

²⁰ <https://www.climatehubs.usda.gov/hubs/southeast>

²¹ <https://www.rma.usda.gov/Federal-Crop-Insurance-Corporation>

Petroleum pollution

Petroleum pollution in marine waters is due primarily to marine vessel spills, accidental discharge from extraction facilities and pipelines, and natural seeps from oil-rich marine deposits. Natural seepage in the Gulf of Mexico is estimated at 140,000 metric tons (mt) per year (range: 80,000–200,000 mt/yr), with approximately half of that amount deriving from the northern Gulf of Mexico (132). The low density of petroleum places it into the biologically productive sea surface and upper water column, co-locating it with offshore pens. Although volatile compounds evaporate from oil slicks at the surface, oil droplets emulsified by wave action and oil bound to suspended sediments become less buoyant, remaining suspended in the water column or drifting to the seabed (133). If a dispersant is applied to the spill, the oil and dispersant form an aggregate (also called marine snow) that can accelerate microbial degradation by increasing the spill's surface exposure (133). In addition to containment and physical removal of oil spills, the importance of degradation by in situ bacteria in the Gulf of Mexico, most likely primed by natural seepage, was demonstrated in the *Deepwater Horizon* oil spill (134).

The obvious effects of oil spills are well documented; they include killing wildlife, fouling of boats, nets, and lines, and the risk of seafood becoming tainted with an objectionable oil taste. Toxic components of petroleum become bioavailable through ingestion, even by plankton, and these chemicals are capable of causing both short-term acute effects (including death) and long-term chronic problems (135).

Mitigation actions for spills are limited for open water aquaculture. A basic response is to boom cages to prevent contact with an oil slick (136). If sufficient notice is given and the trajectory of the spill plume can be projected, cages can be relocated, stock may be transferred out of the area, or stock could be harvested early. However, relocating cages may pose a risk to stock within the cage. Because movement of cages or stock would be subject to permit requirements, an approved contingency movement plan for oil spill response would speed the process. Cages configured for rapid detachment of mooring and anchor line would also facilitate transfer or movement (137). Avoidance of oil slicks may be possible through the use of sinking cages, especially for shellfish, although this is an expensive investment and does not avoid oil suspended in the water column (136).

NOAA maintains a public, internet-accessible, GIS-based Gulf of Mexico Environmental Response Management Application (ERMA) which uses real-time and static information for rapid visualization of hazardous events in the Gulf of Mexico.²² ERMA is used by emergency responders, and can be used by producers for tracking a hazard and planning a response. Producers can also call the National Response Center for current information on a 24-hour telephone line (800-424-8802).

²²<https://erma.noaa.gov/gulfofmexico/>

Harmful algal blooms

Harmful algae are unicellular phytoplankton, or microalgae, that produce biotoxins with negative effects on animals and humans. Under certain conditions, individual species expand to large numbers, creating a harmful algal bloom (HAB) event. (Some HAB events are also colloquially called “red tides,” although there are visual red tides that are not harmful, and not all HABs are red.) HABs can sicken and kill marine organisms, and frequently result in closures to shellfish harvest due to accumulated biotoxins (e.g., domoic acid, saxitoxin). Although there are a large number of toxic and potentially toxic marine species in the Gulf of Mexico (see 138, their Table 1), there are five HABs that receive the most attention in the region.

- *Karenia brevis* blooms are a leading concern in the Gulf of Mexico from Florida to Texas, because they can kill marine fish, birds, turtles, and mammals, and can cause respiratory and eye distress in humans through production of brevetoxin, a neurotoxin very similar to ciguatera (reviewed in 139). This HAB is closely monitored due to its impacts on humans, and NOAA maintains multiple online forecasting tools for *K. brevis* in the Gulf of Mexico.²³
- *Pseudo-nitzschia* spp. occupy a broad range of habitats from the coast to open offshore waters, and blooms are associated with increases in dissolved nutrients, higher water temperatures, and abundant light levels. This microalga produces domoic acid, a potent neurotoxin that causes gastrointestinal distress and neurological damage (e.g., seizures, weakness, memory loss), and can be fatal. High abundances and seasonal fluxes of *Pseudo-nitzschia* spp. on the Gulf of Mexico shelf have been known for decades (e.g., 140), and domoic acid can be transferred through commercially and ecologically important finfish species such as Florida pompano (*Trachinotus carolinus*), striped anchovy (*Anchoa hepsetus*), and Gulf kingfish (*Menticirrhus littoralis*; 141).
- *Dinophysis* spp. blooms produce produce okadaic acid and dinophysistoxins that cause gastrointestinal distress (diarrhea, vomiting, pain) in humans. The first shellfish harvest closure in the United States due to a *Dinophysis* bloom occurred in the Gulf of Mexico in 2008, triggering a need to understand ecological drivers of bloom dynamics for detection and early warning (142).
- *Pyrodinium bahamense* var. *bahamense*, a harmful alga that appears limited to Florida and Costa Rica coastal waters (143), produces saxitoxin, which is capable of causing paralysis and death. Although *Pyrodinium* saxitoxin has not yet demonstrated effects in wildlife, saxitoxin from other HAB species (e.g., *Alexandrium* spp.) is transmitted through the marine food web to top-level organisms and causes mortality in marine birds and mammals (138).
- *Gambierdiscus* spp., which produce ciguatoxin, are the most globally prevalent HABs affecting humans (138). Fish can accumulate ciguatoxin to high levels, and depending on species and tissue burdens, effects range from no toxicity to death (144).

²³<https://coastalscience.noaa.gov/research/stressor-impacts-mitigation/hab-forecasts/gulf-of-mexico/>

Some species can have direct and indirect effects on marine organisms not associated with toxins, such as physical damage to respiratory organs (e.g., gills) and oxygen depletion due to high biomass (138). Blooms of brown-tide species *Aureococcus anophagefferens* and *Aureoumbra lagunensis*, dinoflagellates *Margalefidinium polykrikoides* and *Karenia mikimotoi*, and the raphidophyte *Heterosigma akashiwo* can reduce light penetration in the water column to seagrass beds that are nurseries for many commercially important finfish and shellfish, and the deposition of dead microalgae creates hypoxic or anoxic conditions (138). Additional impacts on marine organisms include reduced growth, feeding behavior changes, or increased susceptibility to disease and parasites resulting from sublethal consequences of toxicity (145).

Southern California Region

Petroleum pollution

Southern California waters support substantial volumes of large vessel traffic, contain numerous oil drilling rigs, and have natural oil seeps. These are the most common sources of petroleum pollution in marine waters, posing a realistic threat to open-water aquaculture. Spills from oil drilling rigs in Southern California have occurred since 1910, with the largest spill to date—approximately 4.2 million gallons—occurring in 1969 near Santa Barbara, then expanding to Pismo Beach in the north and to Mexico in the south.²⁴ Natural oil seeps also constantly release oil, with the total oil seepage for Southern California estimated at approximately 17,000 mt/yr (132).

The hazards and mitigations for oil spills are similar to those described above for the Gulf of Mexico. ERMA has a Southwest region map covering the entire California coast²⁵ that can be used by producers for near-real-time information for mitigation decisions. The State of California maintains an Office of Spill Prevention and Response (OSPR) through the California Department of Fish and Wildlife.²⁶ In addition to providing a spill reporting service (800-852-7550), OSPR coordinates with federal agencies during active spill responses.

Wildfires

Wildfire is a routine event in California, with a 22-year average of 8,329 fires burning 1,002,822 acres annually.²⁷ Large fires release significant clouds of smoke and ash, and these aerosols contain large amounts of nutrients, including phosphorous, nitrogen, and iron. Assessment of the 2019–20 wildfire plumes identified a relationship to anomalous phytoplankton blooms in Australian waters and the South Pacific, likely due to a fertilizer effect from aerosol nutrients (147). Rapid phytoplankton blooms can attract large numbers

²⁴ <https://response.restoration.noaa.gov/about/media/45-years-after-santa-barbara-oil-spill-looking-historic-disaster-through-technology.html>

²⁵ <https://erma.noaa.gov/southwest>

²⁶ <https://wildlife.ca.gov/OSPR/About>

²⁷ https://en.wikipedia.org/wiki/List_of_California_wildfires

of planktivorous fishes and mammals, and, depending upon the species present, could result in a harmful algal bloom. Understanding of the impacts of wildfires on ocean conditions is still in the early stages, and producers should be aware of this evolving area of knowledge.

Dichlorodiphenyltrichloroethane (DDT)

Historically, wastewater discharge (including industrial waste), terrestrial runoff, and aerial fallout were primary inputs of DDT into the marine environment in California (148). Between 1930 and the mid-1970s, federal and state authorities approved 14 marine dump sites along the Southern California coast for industrial and military wastes, including DDT.²⁸ The shallow site on the Palos Verdes shelf received an estimated 800–1,000 tons of DDT, and its designation as a Superfund site allows ongoing remediation of contaminated sediments.²⁹ In 2021, researchers documented the discovery of up to 25,000 barrels of containerized DDT waste in the San Pedro Basin near the Palos Verdes site, and analysis showed sediment concentrations up to ~40 times those at the Superfund site (149).

DDT is transferred from lower trophic levels up through the marine food web, and there are even indications of transfer to terrestrial scavengers (150). Although the application of DDT in the United States was discontinued in 1972, the chemical continued to be manufactured at the Montrose Chemical facility in Palos Verdes until the mid-1980s. It continues to be an environmental and biological problem due to the high persistence of DDT and its metabolites.

At this point, DDT poses a more significant seafood safety concern, especially for filter-feeding organisms such as bivalves. Whether there are direct effects on the health and welfare of cultured organisms has not yet been demonstrated.

²⁸ <https://www.epa.gov/ocean-dumping/southern-california-ocean-disposal-site-2-investigation>

²⁹ <https://19january2017snapshot.epa.gov/www3/region9/superfund/pvshelf/index.html>

Workshop Summary: Best Practices for Biosecurity and Disease Management in Marine Aquaculture (12 July 2022)

Background

A virtual workshop was held on 12 July 2022 and included 16 invited participants (see [Table 1](#)). The objectives of the workshop were twofold:

1. To obtain current, relevant information about biosecurity and disease management from professional practitioners in marine aquaculture that could be incorporated into science support documents for the programmatic environmental impact statements (PEISes) for the Gulf of Mexico and Southern California AOA study areas.
2. Provide foundational knowledge on practices, challenges, gaps, and needs that can inform improvements in marine aquaculture, including research planning.

Participants represented the three sectors of marine aquaculture (finfish, shellfish, seaweed/macroalgae), and, in some cases, a participant represented multiple sectors. Geographic representation was relatively homogeneous across the coastal United States, including Alaska and Hawai'i. Participants represented aquaculture businesses, aquaculture research, aquaculture extension services, and aquatic organism health laboratories. The workshop was facilitated by Seatone Consulting (R. Wilson, facilitator; M. Wylie, co-facilitator).

Prior to the workshop, participants provided anonymous answers to the following questions:

1. What are the three greatest vulnerabilities to disease/pathogens for your area of aquaculture?
2. What do you think are the most effective measures to minimize each of those three vulnerabilities?
3. Although there are ideal measures for minimizing vulnerabilities, they often are difficult or impossible to implement in the real world. Are there more practical measures that can be applied instead?

Responses ($n = 52$) to these pre-workshop questions aligned into five categories:

1. **Human actions:** Nearly all of the responses in this category (38% of total responses) included the introduction and spread of pathogens through imports, transfers, shipping, and visitors, through both legal and illegal actions.

- **Pathogens:** This category (31% of total responses) included specific pathogens such as OsHV-1 or ISAV, and nonspecific pathogens such as emergent pathogens or ectoparasites in general.
- **Environment and infrastructure:** Responses in this category (21% of total responses) were evenly divided between water biosecurity and climate change-related stressors.
- **Knowledge:** Principal concerns in this category (6% of total responses) were lack of information about emergent pathogens, utilization of newer host species, and lack of trained pathologists.
- **Drugs and chemicals:** This category (4% of total responses) included lack of sea lice medications and concern about antibiotic resistance.

Because more than two-thirds of pre-workshop responses involved human actions and pathogens, the workshop discussion focused on these two topics, although overlap with the other areas was expected and did occur.

In the workshop, participants provided cross-sector observations about impediments to addressing biosecurity and disease management, perceived research needs and beneficial improvements to communications, shared information, and disseminated best practices.

To explicitly identify sector-specific topics, participants provided expanded information on current practices, current challenges, and needs to address issues posed by human actions and pathogens. Their information was documented in both a written (notes on an online digital whiteboard) and verbal mode.

Cross-Sector Observations

Impediments to biosecurity and disease management

A main barrier is the lack of programs that are directed toward research and implementation of biosecurity and disease management. This may be related to a need to improve the linkage between industry and academia, where much of the research is conducted. USDA APHIS has a pathogen-specific pathway for best management practice (BMP) development that is based on ISAV and that may be a useful BMP development template for other pathogens.

Another barrier across the sectors is the availability of qualified staff for developing and implementing biosecurity plans, providing training to producer staff, investigating health and disease problems, and diagnosing diseases. This area of expertise is not part of typical operational staffing requirements and is costly for individual growers to assume. In terms of diagnostics, there is limited qualified pathology laboratory capacity for timely and accurate testing; this is further compounded by lack of some consistency in lab protocols and interpretations, especially for shellfish. Furthermore, there is concern about ensuring that there is a positive and enabling atmosphere around implementing biosecurity measures and reporting diagnoses, rather than one that penalizes growers for honesty.

The one challenge faced by all sectors is the uncertainty of changing environments in open-ocean culture and ongoing climate change. There is less parameter control in open-ocean culture than in onshore or even nearshore culture. Furthermore, marine aquaculture is likely to involve cultivation of novel species, for which there is little information about health requirements and disease potential. The absence of an indemnification program for aquaculture is due to the lack of risk assessment and historical data required for actuarial calculations, meaning that growers must assume all of the financial risks.

Major research needs

Cited research needs were distributed into six categories: host physiology and disease resistance, host genetics/epigenetics, pathogen knowledge, testing and diagnostics, disease prophylaxis and treatment, and applied research in general.

Participants agreed that rearing healthy stocks is the first defense against disease, especially opportunistic infections often caused by organisms that are commonly present in the environment. However, there is less research on understanding stressors and their effects on aquaculture organisms than there is on specific diseases. Improved understanding of how to rear and monitor robust host species for health can prevent many disease situations. This type of physiological research needs more support, and is logically coupled to selective breeding for resilience to multiple stressors and for disease resistance. One cautionary note about selective breeding for disease resistance is the potential unintended consequence of greater susceptibility to other diseases that were not the target of the breeding program, or other genetic deficiencies.

The mechanistic basis of selective breeding involves host genetics, epigenetics, and transgenerational effects. While host genetic research is common, awareness of epigenetic effects or transgenerational effects is poorly understood. This type of research might be included in stress physiology research, which would allow phenotypes to be coupled with epigenetic features. For red macroalgae, research on sexual reproduction is needed to help address the disease risks associated with repeated cloning—the industry’s current reproductive approach.

Information about pathogens is fundamental to risk reduction, but current research tends to focus on a handful of well known pathogens. Marine aquaculture is expanding the number of host organisms under cultivation and, consequently, of their associated pathogens, including those that are opportunistic. Research into the fundamental biology, life cycles, and ecology of pathogens and parasites is needed beyond the well studied organisms. Surveillance of known pathogens and parasites in wild marine stocks is only anecdotally conducted, and more systematic surveillance is needed to better understand their ecology and inform risks to aquaculture.

Pathogen testing for diagnostics does receive considerable research attention, although most reported methods can be technically challenging, expensive, and/or unvalidated. Research emphasis on the development of rapid, validated field testing options would provide better support for biosecurity and disease management. Currently, histology is the

one method that allows for detecting multiple pathogens, although histological training is a major investment. Alternative methods for detecting multiple pathogens would be more efficient than single-pathogen detections.

Prophylaxis and treatment of diseases are the most commonly used tools for disease management. For organisms with inducible, sustained immune responses, vaccines are an extremely useful approach. Greater support for vaccine research and development, including industry-scale delivery systems, would be very beneficial for marine finfish aquaculture. Therapeutics are often the only recourse (aside from culling) for managing diagnosed diseases, but identification, safety and efficacy testing, and approval for use is slow. This is due in part to the small size of the marine aquaculture sector and difficulty in identifying drug sponsors.

All of the above points fit into a broader philosophy of greater emphasis on applied research and technology transfer to industry. This could be fostered through funding programs that are informed by input from industry. In addition, funding periods beyond one or two years are likely to be more beneficial, because many issues cannot be addressed in such a short time frame, and outcomes of rearing cycles typically exceed two years.

Improvements to communications, information sharing, and dissemination of best practices

Communications are an important component of an effective biosecurity network, both within a producer's operation and regionally among producers. Participants identified several improvements in communication that can be beneficial for marine aquaculture.

For establishing research and development goals, a forum for regular (annual, biannual) meetings of industry, research, and regulatory communities can mutually improve understanding of the needs and feasibility of disease and biosecurity issues. In addition to meetings, researchers could be exposed to the realities of commercial production through site visits or participation in production activities on working farms.

For developing biosecurity strategies and BMPs, there is guidance provided by national voluntary programs such as the Comprehensive Aquaculture Health Program Standards (CAHPS) or international programs such as Ocean Best Practices.³⁰ However, successful regional industry association programs could serve as models for other regions. Since 1992, the Maine Aquaculture Association has progressively developed BMPs that rely upon comprehensive health surveillance, third-party biosecurity audits, pathogen-specific action plans, area management agreements, site rotation and fallowing, farm and area biosecurity plans, third-party certifications, and a cooperative communication system. The advantages of this and other trade association programs are their high relevance to industry needs and the necessary agreement by producers for implementation and compliance.

³⁰<https://www.oceanbestpractices.org/>

For building trust with groups within industry and outside of the industry–research–regulation community, a system of reporting disease detection, outbreak, and response may be important. Public reporting is obviously a sensitive area for commercial growers, and this topic deserves a robust discussion about boundaries and implementation by the aquaculture community.

Sector-Specific Observations

Participants provided sector-specific information through systematic response on current practices, challenges, and needs for human actions and pathogens that contribute to biosecurity and disease vulnerability. These responses were classified into nine categories:

- Regulation and policy.
- Pathogen knowledge.
- Host knowledge.
- Environmental knowledge.
- Personnel.
- Testing capacity.
- Infrastructure.
- Communications.
- Finance.

The categories are bolded in the discussions below.

Human actions

Current practices

Across aquaculture sectors, current management of issues caused by or related to human actions (Figure 3) relies heavily on existing **regulation and policy** at multiple jurisdictional levels. These measures include state and regional regulations restricting access and movement of broodstock, seed stock, and live organisms; within-farm best management practices, personnel training, audits, and annual internal plans; farm certifications by nongovernment organizations; area management agreements; guidance and assistance from the Interstate Shellfish Sanitation Conference (ISSC) for seafood safety; and federal (USDA) pathogen-specific programs. **Knowledge of pathogens, hosts, and environments** is a second important existing capability for managing disease and biosecurity, which includes pathogen surveillance, proper shell disposal, water quality monitoring and forecasting, and expanding knowledge of new species being cultured. **Testing capacity** (use of histology for broad-spectrum detection of disease), **infrastructure** (use of engineering and structural controls for risk management), and **communication** (industry measures encouraging better biosecurity) are additional important tools for managing disease and biosecurity.

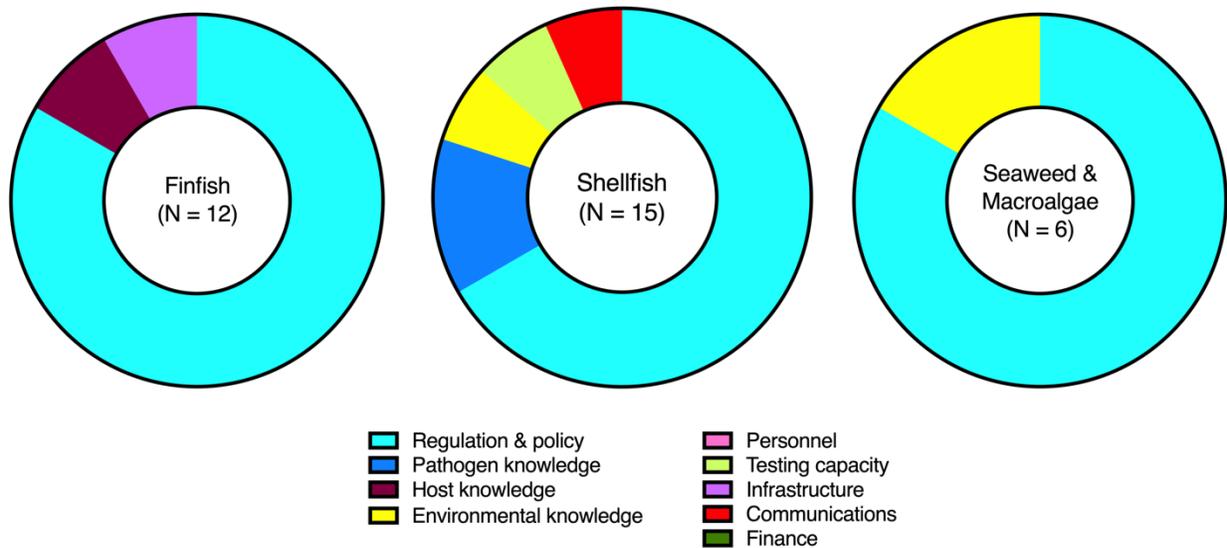


Figure 3. Plots displaying current practices used to manage human actions in disease and biosecurity by aquaculture sector.

Challenges

The current challenges in managing disease and biosecurity are more broadly distributed across the categories and varied among the sectors (Figure 4).

- For the finfish sector, **testing capacity** (shortage of approved or qualified facilities), **regulation and policy** (lack of clarity of authority, inconsistent regulations), and finance (costs of implementing biosecurity) were major challenges. Additional difficulties for the finfish sector are **pathogen and environmental knowledge** (inadequate data on pathogens in wild populations, insufficient monitoring and forecasting tools) and **personnel** (shortage of trained staff).
- For the shellfish sector, challenges were relatively evenly distributed over the categories. **Environmental knowledge** (poor forecasting models, unknown risks of open-water aquaculture, poor understanding of interactions between biology and ecology) is a leading concern. **Pathogen knowledge** (inadequate surveillance of farmed and wild populations), **personnel** (lack of veterinarians and pathologists with shellfish disease expertise), **testing capacity** (shortage of qualified or certified testing labs), and **communication** (poor communication on variable live animal movement regulations across states) are also major challenges. For **regulation and policy** at the federal level, there is concern about live shellfish movements across northern and southern (non-European Union) borders.
- Challenges for the seaweed/macroalgae sector are divided between **regulation and policy** (inappropriate application of finfish and shellfish regulations), **host knowledge** (lack of information about aquatic plant health and disease), and **infrastructure** (lack of cold storage facilities).

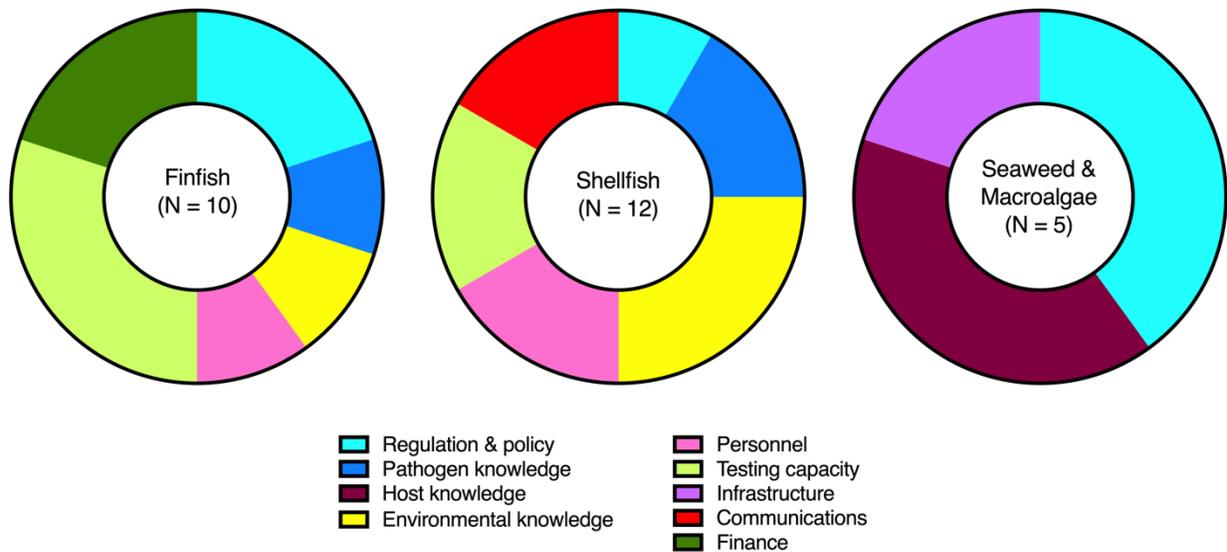


Figure 4. Plots displaying current challenges to managing human actions in disease and biosecurity by aquaculture sector.

Needs

Aside from the category of **policy and regulation**, the needs for good disease management and biosecurity vary across the aquaculture sectors, possibly a reflection of the diversity of challenges (Figure 5). Explicit **policy and regulation** needs include implementation of CAHPS; clarity of regulatory agency roles and responsibilities; centralized and harmonized resources for regulations and practices; specific and common best management practices with development of generic templates for assessing farm risks; qualitative risk assessments based on expert opinions; and better governmental awareness of issues. Beyond this category, the sectors have relatively different needs.

- For the finfish sector, **personnel** needs include better workforce development and partnerships, including training incentives, and improvements in **environmental knowledge** such as aquaculture-specific monitoring and forecasting.
- For the shellfish sector, **personnel** (histopathology training for nonpathologists, increased number of shellfish disease-competent veterinarians) and **communication** improvements (expanded extension services and education, better regional networking among growers) are important for better disease management and biosecurity. Additional needs are better **environmental knowledge** (aquaculture-specific monitoring and forecasting), expanded **testing capacity** (increased number of certified or qualified testing labs), and strengthened **infrastructure** (more redundancy of local seed production).
- For the seaweed/macroalgae sector, expanding **host knowledge** is a substantial requirement, including research on sexual reproductive strategies for red algae and strengthening collaborations with international expertise. Better **pathogen knowledge** (surveillance of wild stocks) and improvements in **personnel** (increased number of aquatic plant health professionals) are needed for this sector.

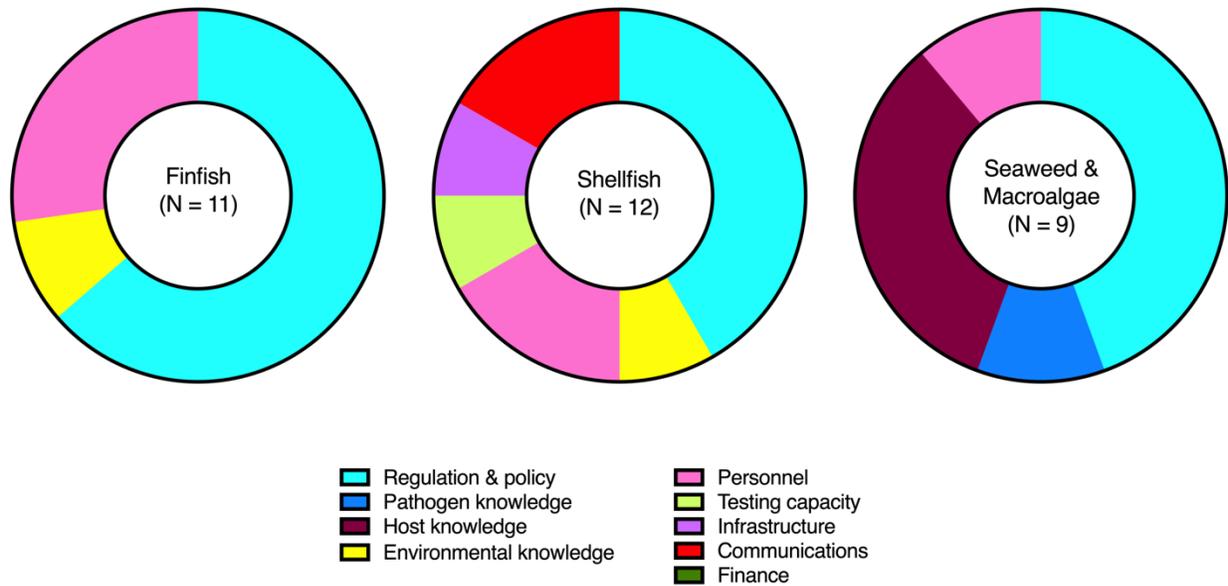


Figure 5. Plots displaying needs for improved management of human actions in disease and biosecurity by aquaculture sector.

Pathogens

Current practices

Regulation and policy and **host knowledge** are the most commonly cited mechanisms across the aquaculture sectors for managing pathogens and disease (Figure 6). Regulation and policy tools range from government rules (existing state and federal requirements for imports and live animal transfers; USDA pathogen-specific general health programs such as CAHPS), to actions developed and implemented by industry (best management and biosecurity practices, especially in hatcheries; strong health programs including disease testing and mortality monitoring; good staff training), to actions involving third-party certifications and audits. **Host knowledge** is widely employed by all three sectors to produce robust organisms through low stress and trauma rearing, use of vaccines, employing breeding programs for disease resistance, and managing spatial blocks for seed exchange between regions and states. For finfish, **infrastructure** is a current practice that includes secure incoming water supplies for recirculating aquaculture systems (RASes) and farm site selection. For shellfish, **pathogen knowledge** through surveillance and good **communication** between producers and regulators are additional important current practices.

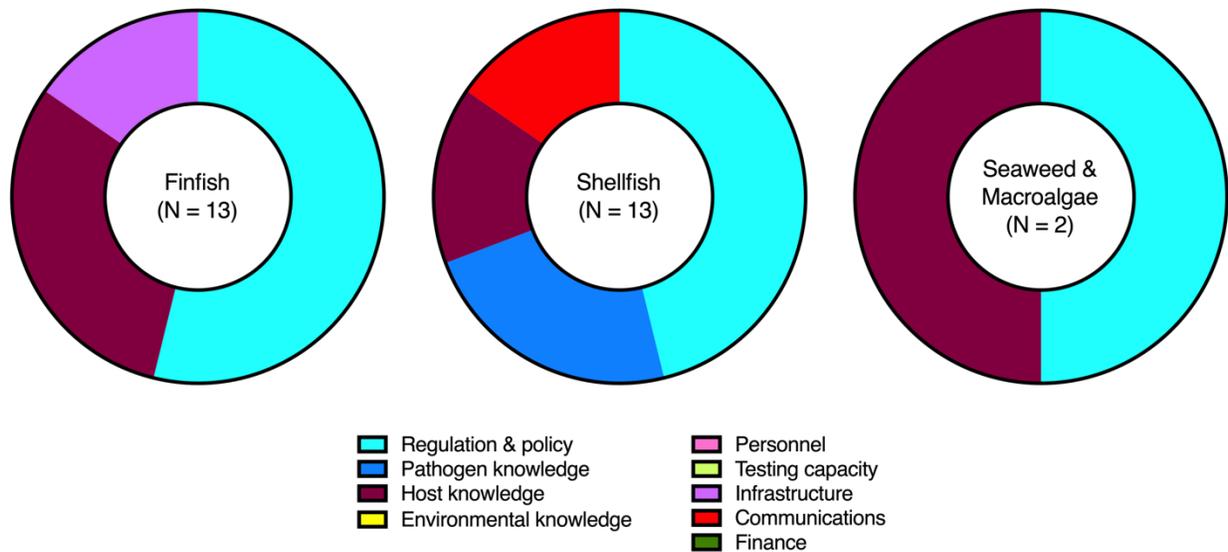


Figure 6. Plots displaying current practices used to manage pathogens in disease and biosecurity by aquaculture sector.

Challenges

Although the challenges for disease management and biosecurity varied across the sectors, **pathogen knowledge** and **host knowledge** were cited as the majority issues (Figure 7).

- For the finfish sector, the challenges in **host knowledge** are the slow pace and cost of vaccine and therapeutant development and licensing (including investigational new animal drug [INAD] approval) and the relatively low level of support for health and disease research (breeding programs, diet research, low number of disease challenge facilities). Gaps in **pathogen knowledge** include understanding risks associated with opportunistic pathogens, insufficient data on pathogens in wild populations, and risks of pathogen introduction through feed. Challenges in **regulation and policy** are the lack of consistent standards for interpreting positive laboratory findings, lack of regulation enforcement, and the lack of mandatory federal disease and biosecurity programs. For open systems in the oceans, the **environmental knowledge** about ecological perturbations such as red tides is low. The absence of an indemnification program for aquaculture losses is an important **finance** challenge for the industry.
- The shellfish sector has the greatest diversity of challenges associated with pathogens. Gaps in **pathogen knowledge** include the lack of consistent, high-quality surveillance of both farmed and wild animals, and insufficient knowledge about emerging pathogens, pathogen life-history cycles, and vectors. **Host knowledge** challenges include little or no research and development on rearing healthy stocks, lack of standardized tests for stock health (e.g., stress tests), poor integration of pathology into breeding programs, and low understanding of the role of environment on disease. The paucity of veterinarians and health professionals with expertise in shellfish health and disease is an important **personnel** problem, and **testing capacity** is impeded due to inadequate lab facilities, lack of rapid testing, and unvalidated molecular assays for shellfish diseases. The important **infrastructure**

issue is the lack of disease-free areas for rearing and for holding broodstock. The sole **regulation and policy** challenge around pathogens is poor information about the European Union import program, which may be related to a **communication** issue around the level of trust among producers, regulators, and diagnostic laboratories.

- The dominant challenges about pathogens for the seaweed/macroalgae sector are about **pathogen knowledge** (lack of knowledge and expertise about diseases and pathogen life cycles; no processes to identify and track pathogens) and **host knowledge** (disease vulnerabilities in using clones for tropical red algae; limitations in managing epibionts of kelp). A **regulation and policy** issue for this sector is the inappropriate application of regulations that were developed for other sectors. Because the seaweed/macroalgae industry is fairly new and relatively smaller, **finance** burdens are relatively higher.

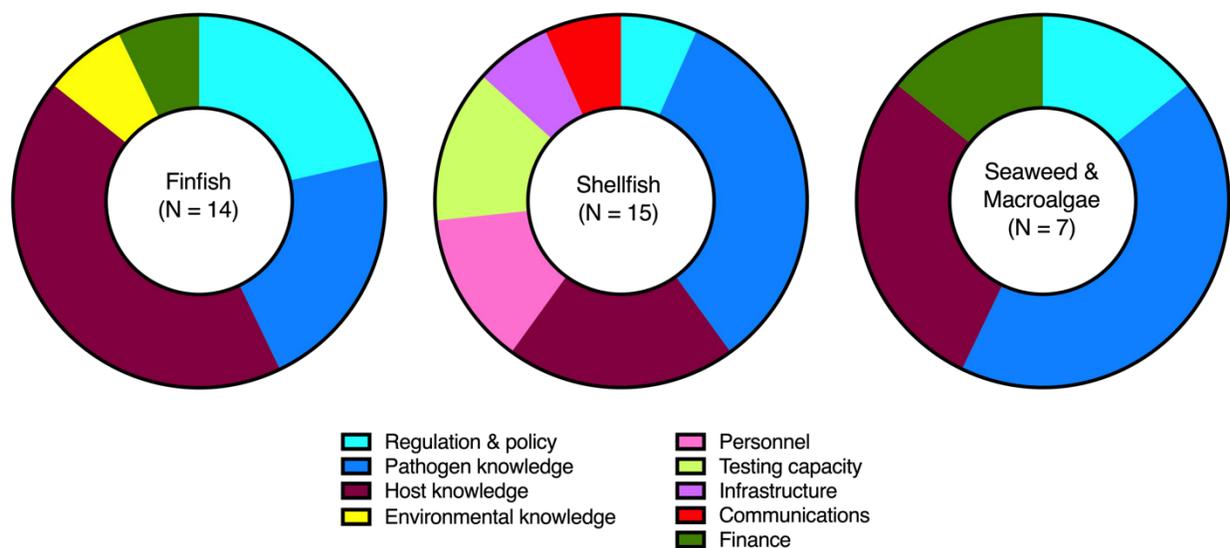


Figure 7. Plots displaying challenges in managing pathogens for disease and biosecurity by aquaculture sector.

Needs

The needs vary considerably across the marine aquaculture sectors, and each sector has a diversity of needs (Figure 8).

- For the finfish sector, **host knowledge** is the largest category, and the topics include a larger array of approved therapeutants, faster development and approval of vaccines, reductions in the costs of INADs, and a national-level research and development program (including disease-resistant breeding) for promising finfish species. There are also needs in **pathogen knowledge** (research on opportunistic and production-related pathogens), **environmental knowledge** (better ocean epidemiology models), and **infrastructure** (improvements in feed certification). A **finance** issue is the current pattern of funding opportunities for only one or two years, which is unrealistic for fostering meaningful progress in such a short time frame. In the **regulation and policy** area, there are needs to clarify agency authority, to eliminate overlap or duplication of authority, and to implement CAHPS.

- For the shellfish sector, **pathogen knowledge** and **communication** are the main needs. **Pathogen knowledge** needs include information for developing pathogen response plans, especially for high-risk pathogens; more disease ecology research; better ocean epidemiology models; and measures to prevent pathogen transfer in frozen products. Development of assessments to determine whether robust shellfish are being produced is a **host knowledge** need. **Personnel** needs include increasing shellfish-specific expertise among veterinarians and aquatic animal health professionals, and the development of a shellfish disease expert network. **Testing capacity** can be improved by increasing the number of certified or qualified testing labs, including the use of smaller, regional labs. Better control of shell disposal, including public education, and improved interactions within the industry (across regions) and between industry and testing laboratories are **communication** needs. **Regulation and policy** needs for the shellfish sector include harmonized requirements for stocking and moving animals, support for the Regional Shellfish Seed Biosecurity Program (RSSBP), and better surveillance requirements (possibly including audits) for farmed stocks.
- Seaweed/macroalgae sector needs are dominated by **host knowledge**, **infrastructure**, and **communication**. The primary impetus for these needs is the overall low level of information available for a relatively young industry. **Host knowledge** needs are research on sexual reproductive strategies for red algae, and research on host life cycles and culture methods, including application of molecular techniques. Baseline information about which pathogens are present is an important **pathogen knowledge** need, and an increase in the number of aquatic plan health professionals is needed for this sector’s personnel. Improvements to **infrastructure** include identification of beneficial inocula for nursery seed establishment of specific pathogen-free (SPF) lines, and tissue culture sources for the primary commercial species. For **regulation and policy**, rules for movement of seed based on data, rather than hypothetical risk, are needed. Needed improvements in **communication** are collaboration with international expertise and a state-of-the-art workshop resulting in a monograph or manuscript on the status of the field.

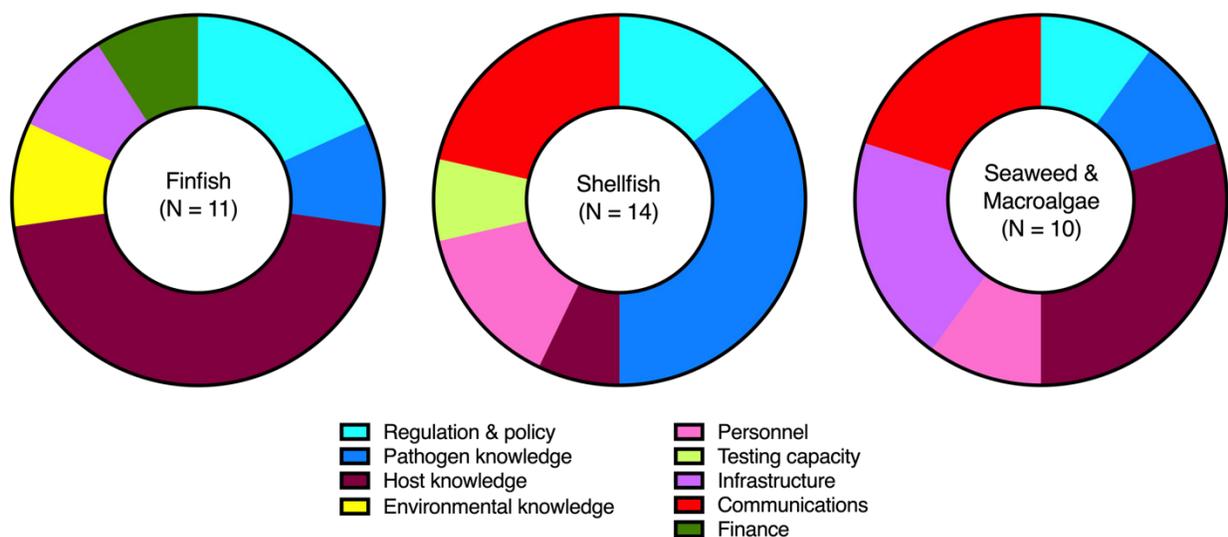


Figure 8. Plots displaying needs in managing pathogens for disease and biosecurity by aquaculture sector.

Key Points and Conclusions

The workshop was an opportunity to bring together active marine aquaculture practitioners with a focus on actions and topics directly relevant to biosecurity and disease management. The participants raised issues that covered a range of disciplines and reflected the specific state of maturity for respective sectors. However, there were overarching issues involving a need for regulatory and policy clarity and appropriateness; expertise in disease detection and diagnosis; support for research on rearing robust organisms; monitoring pathogens and environmental conditions; availability of standardized, practical tools for biosecurity planning and management; and using communication to facilitate research goal-setting and elevating trust within the aquaculture community.

It is important to note that the number of workshop participants was small, and these reported observations are not intended to reflect the entire industry. We made considerable effort to ensure broad representation, and the final participant group was distributed geographically around the U.S. coasts (7 East Coast, 3 West Coast, 4 Hawai'i, 1 Alaska), across the aquaculture sectors (10 finfish, 6 shellfish, 4 seaweed/macroalgae; some participants represented more than one sector), and by organization (7 industry, 4 academia, 2 NGO, 2 government). A potential utility of the results of this workshop is to guide and inform a deeper query of marine aquaculture practitioners, researchers, and regulators on relevant biosecurity and disease management issues.

The observations from this workshop were incorporated into the information provided throughout this technical memorandum. Furthermore, the workshop results can serve as a basis for future assessment of investments in research and development in support of U.S. marine aquaculture.



Table 1. Invited participants to the workshop.

Name	Organization	Participation*
Sebastian Belle	Maine Aquaculture Association	1
Tal Ben-Horin	North Carolina State University	1
Dave Bushek	Rutgers University	1
Ryan Carnegie	Virginia Institute of Marine Science	1
Mike Congrove	Oyster Seed Holdings	3
Joth Davis	Pacific Hybreed, Hood Canal Mariculture, Bay Shellfish, Puget Sound Restoration Fund	3
Mark Drawbridge	Hubbs SeaWorld Research Institute	1
Erin Ewald	Taylor Shellfish	1
Cem Giray	Salmonics	1
Melissa Good	Alaska Sea Grant	3
Kathleen Hartman	USDA Animal and Plant Health Inspection Services	1
Maria Haws	Pacific Aquaculture and Coastal Resource Center, University of Hawai'i Center of Excellence for Sustainable Aquaculture	1
William Keleher	Kennebec River Biosciences	1
Tyler Korte	Blue Ocean Mariculture	1
Jim Parsons	Jamestown Seafood Company	3
Alex Primus	Hubbs SeaWorld Research Institute	1
David R. Russell	Maine Department of Marine Resources	2
Neil Sims	Ocean Era	1
Tori Spence	National Marine Fisheries Service Pacific Island Region	1
Tiffany Stephens	Seagrove Kelp Company	1

*1 = attended workshop, 2 = unable to attend but provided responses, 3 = unable to attend.

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