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Aqua Aquaria India 2017

14th - 16th May 2017, Nehru Maidan
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Provisional guidelines for bivalve farming in India with emphasis on meeting hygiene and public health requirements

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1. Introduction

Bivalve molluscs such as clams, mussels, oysters and scallops are prime seafood that contribute to capture and aquaculture production in many countries. Global bivalve aquaculture production has increased two-fold from 7.08 to 14.72 million tons during 1995-2015. While production has increased, the share of bivalves entering the international trade is relatively small, due to the stringent regulations on their imports associated with the food safety issues in major markets. The regulatory regime for bivalve products, under live or processed category, varies with the importing country. For instance, though EU is one of the main markets accounting for one-third of the total bivalve trades, only 13 non-EU countries are authorized to send live bivalves to the EU markets. The rapid alerts of non-compliance of bivalve mollusc/products in border inspection posts in EU Rapid Alert System of Food and Feeds (RASFF) indicates the magnitude of the problem associated with bivalve imports in EU (Fig.1).This, stresses strict sanitary control of such type of seafood, restricting many nations from penetrating into export markets beyond their provinces. Accordingly, the share of bivalves (clams, oyster, mussels and scallops) in the international export trade of fish and fishery products (estimated at US$ 139.1 billion)in 2013 is only 1.53% in quantity and 2.15% (US$ 2.997 billion) in value (FAO, 2017).

In India, farming of oyster and mussel is an expanding sector in coastal and estuarine waters since late 1990's. Bivalve farming is practiced in near shore waters under the state and village jurisdiction. These near shore farming areas provide suitable environmental conditions for bivalve growth, ready access to the farming structures, facilitate effective management, stock protection, transportation and thereby reduce the operational expenditure in farming activities. On the contrary, these near shore farming activities are subjected to extensive multi-user conflicts.

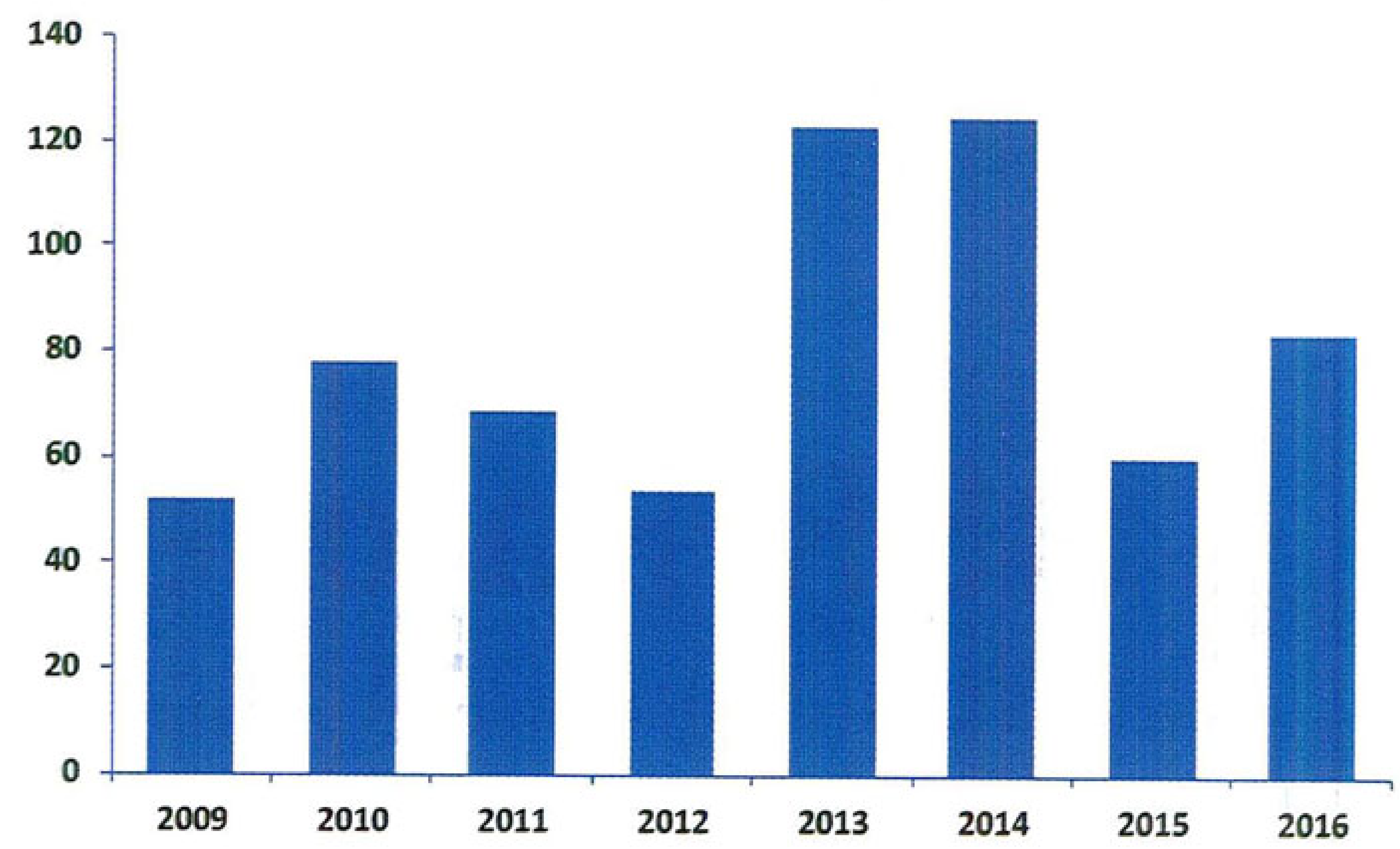

Fig. 1 Rapid alerts of non-compliance of bivalves in EU Rapid Alert System of Food and Feeds (Source: RASFF portal)

Marine bivalves are known as efficient filter feeders, pumping significant amounts of water (e.g., tens to hundreds of litres each day by each mussel/oyster) for trapping the food particles (Shumway and Rodrick, 2009). The near shore coastal areas receive contaminants from land runoff, sewage, industrial effluents as well as from a host of anthropogenic activities and natural events. Due to the proximity of bivalve farming sites to the coast, they are susceptible to point and non-point sources of pollution. The contaminants are accumulated through both dissolved phase uptake and particle ingestions by filter feeders. Since bivalves are filter feeders, when they are exposed to chemical or biological pollutants, they have a high risk of becoming contaminated and tend to become unsafe as food.

To minimize the probable risk of consuming contaminated bivalve, many countries have implemented shellfish (bivalve) sanitation programmes that sets standards for bivalves or bivalve growing/harvesting waters or both. Regular sanitary test ensures that the bivalves are safe for human consumption.

2. Bivalve Mariculture in India

Bivalve aquaculture meet the growing need for quality seafood from the underutilized near shore waters. During the past 20 years, bivalve production from this sector has steadily increased along the southwest coast of India, especially in the state of Kerala. With appropriate management, this trend is projected to continue in the near future. Besides seafood, this environmentally sustainable industry provides direct and ancillary employment on and off the aqua farms and contribute to rural development.

Recent advances in the sector indicates adaption of farming techniques to local context, resulting in adoption and horizontal spread of this technology in the states of Karnataka, Goa and Maharashtra. The green mussel Perna viridis and the Indian backwater oyster Crassostrea madrasensis contributes to the farmed bivalve production in India. During 2009, the bivalve production reached nearly 20,000 tons with India figuring among top ten bivalve farming countries in Asia (Mohamed et al., 2016).Recent mussel and oyster production in 2015 respectively places the nation at 13th and 17th position globally (Fig. 2).

Although such significant mariculture developments have taken place in India, they have happened without the support of proper rules and regulations to govern the practices in open water bodies. Earlier, Mohamed and Kripa (2010) have suggested a set of guidelines for open water leasing for all mariculture activities in the country, and these need to be urgently put in place by concerned local bodies and government for sustainable development of bivalve farming. This is particularly important, when unscientific farming practices and environmental stress can lead to proliferation of protozoan parasites which can potentially lead to mortality of farmed stocks (Sanil et al., 2012).

Besides farming, mussels, oysters and clams are traditionally harvested since ancient times for food and shells by exploiting their regional distribution in coastal areas and/or estuaries. The bivalve trade in India for gastronomic purpose either from capture and culture production is regionalized primarily around the region along the west coast of India, except in high-end restaurants. However, bivalve shells and sub fossil shell deposits are extracted for industrial use all along the east and west coast of Indian subcontinent.

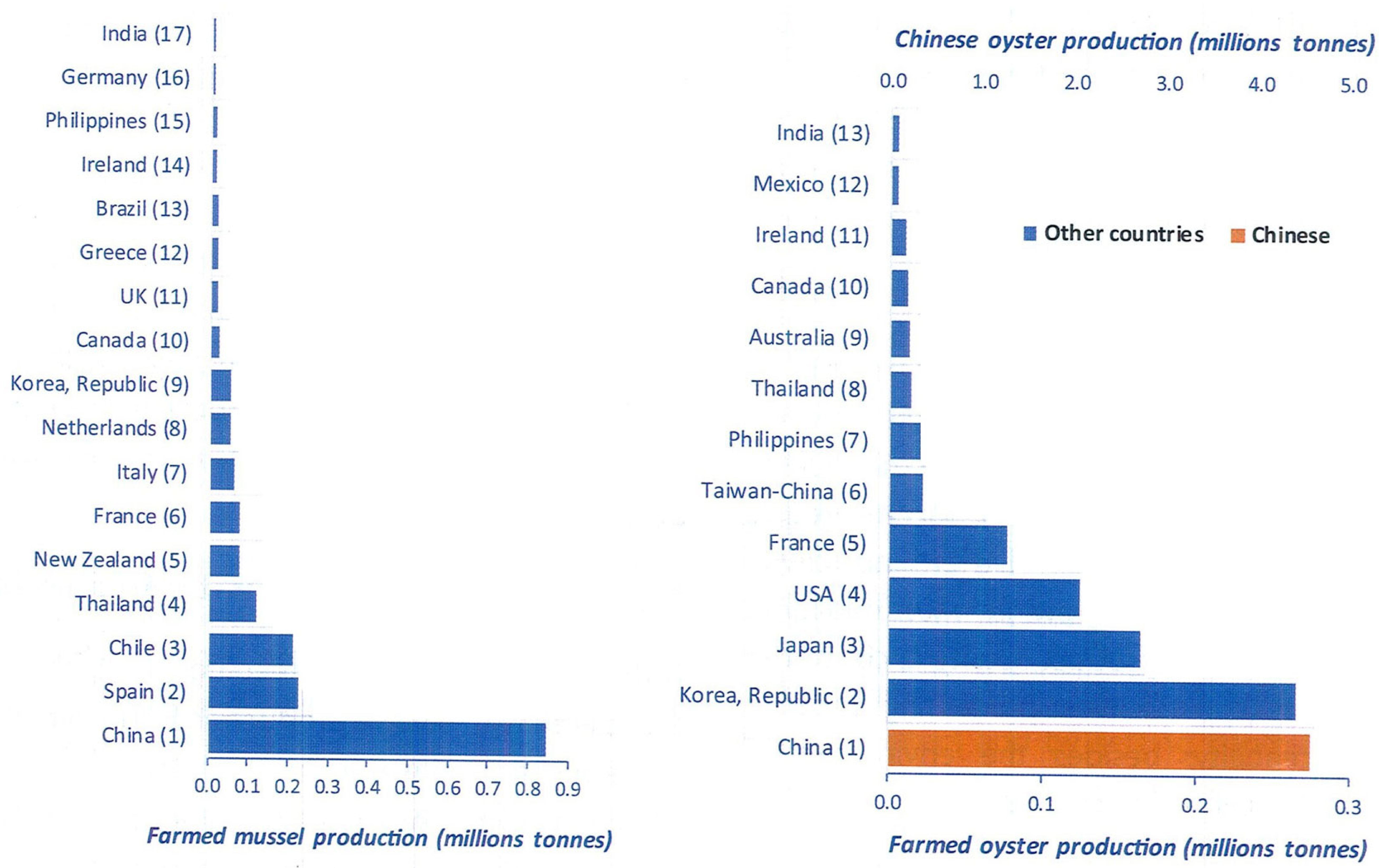

Fig. 2 Major farmed bivalve producing countries in order of ranking in 2015 (figures in parenthesis indicates rank)

3. Bivalve (Shellfish) Sanitation Programmes

Several countries involved in bivalve growing/ harvesting have an effective sanitation programme in response to many disease outbreaks associated with the consumption of contaminated shellfish. The National Shellfish Sanitation Programme (NSSP, US FDA) of the USA and the Canadian Shellfish Sanitation Programme (CSSP) were developed as early as 1925. The NSSP 'Guide for the Control of Molluscan Shellfish' sets forth the principles and requirements for the sanitary control of shellfish produced and shipped in interstate commerce in the United States. It provides the basis used by the Federal Food and Drug Administration.

Similarly, the European Union (EU) establishes compulsory quality criteria for EU countries' shellfish waters to safeguard certain shellfish from the harmful effects of discharges of pollutants into the seas by Directive 2006/113/EC on the environmental quality of shellfish waters.

In Australia, the Australian Shellfish Quality Assurance Programme (ASQAP, 2004), modelled on the US NSSP is used for classifying the shellfish growing areas of Australian waters. Similar programmes operate in Japan and in several European countries.

Certain programmes incorporate a hybrid and tissue standards, to incorporate both food product and environmental health components. New Zealand has been highly successful in utilizing both NSSP and E.U. regulatory components to form a hybrid system that meets the requirements of both target markets, allowing universal export opportunities.

4. Status of Bivalve Quality Control in India

In India, a comprehensive quality control regime in line with the systems implemented by other bivalve producing countries is not in place. Hence, strict quality control measures are not mandatory for marketing bivalves in domestic markets. The bivalve farming activity confronts issues in relation to growing water quality & near shore water pollution; product quality & consumer safety; bulk harvest &post-harvest processing; product handling & traceability; besides the environmental impacts and other location specific issues.

Emergence of a new value chain for farm grown live oysters (*Crassostrea madrasensis*) in high-end restaurants in Kerala necessitated fine tuning of depuration protocols for averting consumer health hazards (Mohamed & Kripa 2013). In India, the depuration protocol for commercial scale farming of oysters is standardized by CMFRI and working models are currently in place. The efficiency of depuration of oysters using the fill draw method with high-loading density in tropical conditions was evaluated by enumeration of total coliforms, faecal coliforms, *Escherichia coli, faecal streptococci, Vibrio* spp., and *Salmonella* spp. (Chinnadurai et al., 2014). Depuration resulted in reduction of coliforms and E. coli from levels exceeding NSSP and European Union standards to the compliance limits in 24 h-48 h. The procedure further enabled complete elimination of *Vibrio* spp from the live oyster in 8 h. The placement of oysters in the depuration tank, guided the purging efficiency of gut content in clean water. Besides this, an ultra-pure Depuration Display Unit (DDU) was designed and standardized by CMFRI for oyster purification (Mohamed et al., 2011). The DDU exhibited in restaurants has an appeal to consumers preferring safer live oysters.

Classification of bivalve growing and harvesting waters have been initiated in India (Sasikumar and Krishnamoorthy, 2010; Chinnadurai et al., 2016, Jenni et al., 2015). Accordingly, the oyster growing waters in Ashtamudi Lake and Azhikode Estuary was classified as Class 'B' according to EU standards. This necessitates depuration or relaying to cleaner areas for self-purification in the natural environment over a period of time.

Aspect of bioaccumulation in green mussels from harvesting areas along the Karnataka Coast indicated higher bioaccumulation of faecal coliforms during monsoon; however, the levels were within the safe limits in pre-monsoon and post-monsoon seasons (Raveendran et al., 1990; Sasikumar and Krishnamoorthy, 2010).

5. Hazards Associated with Bivalve Mollusc

Due to the unique filter feeding habit, the main risk associated with bivalve production is the microbiological contamination of waters in which they grow, especially when the bivalve mollusks are intended to be eaten live or raw. During the filter feeding process, the bivalves concentrate contaminants to a higher level than the surrounding sea water. The contamination with bacteria and viruses in the growing area is therefore critical for the end product specification and determines the process requirements for further processing. Gastroenteritis and other serious diseases such as hepatitis can occur as a result from agricultural run-off and/or sewage contamination like enteric bacterial and/or viral pathogens (norovirus, viruses causing hepatitis) or from natural occurring bacterial pathogens (Vibrio spp.) (Shumway and Rodrick, 2009). Another hazard is due to the presence of bio-toxins in bivalve tissue. Bio-toxins produced by some algae can cause various forms of serious poisoning (Table 1). Isolated incidents of (fatalities) shellfish poisoning were reported India from States of Karnataka and Kerala (Karunasagar, et al., 1984; 1998). Chemical substances, such as heavy metals, pesticides, organochlorides, and petrochemical substances may also form a hazard in contaminated areas.

Table 1. Hazards associated with bivalve mollusc consumption (Source: Lee et al., 2008)

Class of hazard		Contaminant
Infections	Bacteria	*Salmonella* spp., *Shigella* spp., *Vibrio parahaemolyticus*, *Vibrio vulnificus, Vibrio cholerae, Listeria monocytogenes*
	Viruses	*Norovirus*, Hepatitis A virus
Intoxications	Chemical	Heavy metals: including Mercury (Hg), Cadmium (Cd), Lead (Pb). Organics: Dioxins, Polychlorinated Biphenyls (PCBs), Polycyclic Aromatic Hydrocarbons (PAHs), pesticides
	Biotoxin	Paralytic shellfish poisoning (PSP), Diarrhetic shellfish poisoning(DSP), Amnesic shellfish poisoning (ASP), Neurotoxic shellfish poisoning (NSP)

6. Monitoring and Quality Control Guidelines

6.1 Basic Requirements for an Effective Sanitation Programme

Bivalve farming operation should have an effective sanitation programme that supervises the farming, harvesting and marketing of safe bivalves for human consumption, since bivalves may represent a risk for public health as potential sources of pathogenic agents and toxic substances. The basic requirements of such a programme include (Canzonier 1988):

1) Organization/s (competent authority) capable of evaluating the quality of the bivalve growing water (classification), monitoring farming and harvesting activities, and providing adequate surveillance of the chain of supply from the point of production to the point of retail (for traceability and quality testing)

2) An administrative system for coordinating the activities of the various organizations responsible for executing the program, both within the producing areas and between the production area and the receiving markets

3) Appropriate legislation empowering the responsible organization to enjoin and prosecute those who breach regulations.

6.2 Classifying Bivalve Farming Areas

Bivalve producing countries must have a competent authority which is responsible for official controls throughout the production chain. The authorities must be empowered, structured and resourced to implement effective inspection and guarantee credible public health and seafood health attestations in the certificate to accompany fishery products that are destined for the EU/ other importing countries.

Setting standards for responsible aquaculture production by classifying farming areas ensures that the bivalves are harvested from certified waters that meet safety standards. Internationally, bivalve sanitation programs are typically based on either the NSSP of US, which relies on the enumeration of indicator bacteria in water as a measure of fecal pollution, or the EU model, which assesses the exposure of production sites to fecal pollution by determining indicator bacteria present in shellfish tissue (Ogburn and White 2009). These programs apply the principles of prevention of bivalve contamination at source, by growing and harvesting in clean waters and by setting control and processing requirements for more contaminated areas.

Sanitary controls under both systems are supported by a classification of harvesting areas according to the degree of pollution as judged by faecal indicators. This involves a physical survey of the area to evaluate the potential sources of contaminations, which considers the sewage treatment processes, effluent discharges, surface/land runoff, geographical proximity of the contamination source and farming area, tidal cycle, current pattern, bathymetry, seasonal rainfall, tourism, boating , human and animal population in the catchment area.

Bivalve growing area are classified and listed by undertaking a 'sanitary survey ', which consists of a three-fold process:

1) A shoreline survey of the farming area, for confirming potential sources of pollution that may impact water quality, identified through a desk-based study.

2) Water sampling to determine feacal coliform bacterial levels in the marine water; and

3) Analysis of how weather conditions, tides, currents, and other factors may affect the distribution of pollutants in the area.

The most common bacterial indicator used to determine the sanitary quality of coastal waters are the coliforms, specifically Escherichia coli. The sanitary survey provides the basis for determining the designated boundaries of production and relaying areas (Table 2&3) and the sampling plan for ongoing microbiological monitoring by the competent authorities.

The intent of the sanitary survey is to inform the siting of the sampling points, and the timing of sampling with respect to the time of the year (for seasonal farming period), and time relating to potentially contaminating influences, such as tidal effects, rainfall and others. Therefore, sanitary survey should necessarily cover the seasonal cycles in the area, in order that the microbial results that are obtained are representative of the area. The monitoring based on the indicator organisms provides an assessment of the risk of contamination with bacterial and viral pathogens. The area is appropriately classified by the competent authorities based on the results of this assessment. This in turn determines whether the area is suitable for bivalve production and the level of post-harvesting treatment necessary to reduce the risk to a level that is regarded as acceptable.

Table 2. Shellfish harvesting classification criteria based on National Shellfish Sanitation Programme (NSSP, US FDA)

US FDA Classification	Shellfish treatment required	Faecal coliforms (100 ml water)Geometric mean[4]	90th Percentile	Criteria
Approved	None	MPN ≤14/100 ml	MPN ≤43/100 ml	Acceptable water quality; No significant pollution sources.
Restricted	Purification or relaying in an approved area	MPN ≤88/100 ml	MPN ≤260/100 ml	Evidence of marginal pollution
Prohibited	No sanitary survey or conditions for approved/restricted areas not met[2]			Evidence of gross pollution

[1] Values for 5-tube decimal dilution test – different 90 percent compliance values are given for the 3-tube MPN and mTEC membrane filtration tests.

[2] Aspects other than the concentration of contaminants may be used to declare an area prohibited

Nadu. An outbreak of PSP has occurred in Kumbla near Mangalore following consumption of clams, *Meretrix casta* in 1983. In September1997, an outbreak of PSP was reported from Vizhinjam ,Kerala, resulting in the death of seven persons and hospitalization of over 500 following consumption of mussel, *Perna indica*.

If biotoxins are found in the bivalve mollusks tissue in hazardous amounts, the growing area must be closed for harvesting bivalve molluscs until toxicological investigation has made clear that the bivalve molluscs is free from hazardous amount of bio-toxins. The closed status shall be established based on the following criteria (US NSSP):

- The concentration of paralytic shellfish poison (PSP) equals or exceeds 80 µg per 100 g of edible portion of raw bivalves;
- or For neurotoxic shellfish poisoning (NSP), the harvesting of bivalves shall not be allowed when:(i) The concentration of NSP equals or exceeds 20 mouse units per 100 grams of edible portion of raw bivalves; or(ii) The cell counts of causative organisms in the water column exceed 5,000 per liter;
- or For domoic acid, the toxin concentration shall not be equal to or exceed 20 ppm in the edible portion of raw bivalves.
- For azaspiracid shellfish poisoning (AZP), the concentration of azaspiracids shall not be equal to or exceed 0.16 mg/kg (AZA-1 equiv.) in the edible portion of raw bivalves.
- For diarrhetic shellfish poisoning (DSP), the concentration of DSP toxins shall not be equal to or exceed 0.16 mg/kg (OA equiv.) in the edible portion of raw bivalves.

Table 4. United States (US) and European Union (EU) allowable levels for poisonous or deleterious substances in fish and shellfish. S: shellfish; F: fish; ppm: parts per million; DDT: dichlorodiphenyltrichloroethane; DDE: dichlorodiphenyldichloroethylene; DDD: dichlorodiphenyldichloroethane; PCB: polychlorinated biphenyls; PSP: paralytic shellfish poison; DSP: diarrhetic shellfish poison; NSP: neuorotoxic shellfish poison; ASP: amnesic shellfish poison; *WHO (1989) specify 0–60mg per 100g& NSSP specify 0.16 mg/kg; **value for Canada (Todd, 1993). (*Adapted from Shumway (1992) and Huss (1994) (Source: Gosling, 2003)*)

	Allowable level	
Deleterious substance	**US**	**EU**
Dieldrin	0.30 ppm (F, S)	0.1 ppm (F)
DDT, and metabolites DDE and DDD	5.00 ppm (F)	2.0 ppm (F)
PCB	20 ppm (F, S)	2.0 ppm (F)
Mercury	1.0 ppm (F, S)	0.5 ppm (F)
Lead	1.5 ppm (F,S)	2.0 ppm (F)
PSP	80 µg per 100 g meat (S)	80 µg per 100 g meat (S)
DSP	Not specified*	Not specified
NSP brevetoxins	No detectable amount	No detectable amount
ASP domoic acid	20 µg g^{-1} meat** (S)	20 µg g^{-1} meat (S)

For early warning purposes, where appropriate, it is recommended to have a programme present to monitor growing areas for the species of plankton that can produce toxins and to recognize other environmental signals that a toxic event may be developing.

Chemical contaminants, such as heavy metals, pesticides, organochlorides, petrochemical substances are a potential hazard in certain areas. Monitoring of shellfish may also be undertaken for chemical contaminants (EU Directive 2006/113/EC).Harmful chemical substances should not be present in the edible part in such amounts that the calculated dietary intake exceeds the permissible daily intake.

6.5 Purification and relaying

Depuration: Shellfish harvested from class B areas, which are intended for live sale, must be purified. Purification (depuration) procedures are a means of extending the natural bivalve filter-feeding processes in clean seawater to purge out microbial contaminants. Tank based depuration is now widely practiced in many countries including Australia, the UK, France, Italy, Spain and elsewhere. It is, however, less widely used in the US. Depuration periods may vary from 1 to 7 days, with around 2 days being probably the most widely used period. Minimum time periods for depuration are not stipulated in EU Directive 91/492/EEC. From a regulatory aspect, a minimum of 42 hours is specified in the UK and 44 hours in the US NSSP.

Depuration systems also vary and include processes where water is static or changed in batches, flow through systems where seawater is flushed through continuously or recycled through a sterilizer. Depuration has been applied to most bivalve molluscan shellfish species that are sold live.

Relaying:This involves the transfer of harvested animals to cleaner estuaries or inlets for self-purification in the natural environment. Shellfish harvested from EU class C areas, which are intended for live sale, are placed on the market following extended two months relaying. This process can also be used as an alternative to depuration for class B shellfish.

6.6 HACCP approaches

Processing of live bivalve molluscs and/or manufacturing products incorporating such shellfish are required to have a robust Food Safety Management System in place that incorporates Hazard Analysis and Critical Control Point (HACCP) principles and that is operating effectively. The Food Safety Management System must include clear specifications for incoming raw material and finished product, along with procedures and instructions to be followed in the event of a batch of raw material or processed product failing to meet the requirements of these specifications. The US, Canada and EU have proposed a mandatory HACCP-based seafood regulation (FDA, 1994; White & Noseworthy, 1992; EEC, 1993). The HACCP system is based on the recognition that microbial hazards exist at various points in food production, and that measures can be taken to control these hazards.

In addition, there are requirements to ensure that that effective refrigeration controls are in place to prevent these pathogens from growing to levels high enough to cause illness. Additional requirements are designed to ensure that all bivalves are properly tagged, all production and processing facilities are licensed, and that their facilities and operations meet appropriate sanitary standards.

7. Way forward

By monitoring organic& inorganic contaminants, bio-toxins, and by the management of growing areas, the bivalve industry can address the concerns related to the pre-harvest phase and meet the strict requirements imposed by importing nations. In India, bivalve depuration unit developed by CMFRI can be taken as a model for replication in all major bivalve production centres (both wild and farmed harvests). The depuration units should be accessible to the bivalve farmers of the region and must be sited near the farming/ landing centres. Such measures will protect consumers in domestic and international segments. Authorities such as MPEDA should encourage international trading opportunities in bivalve mariculture and fishery products through seminars, trade missions, market intelligence and market development activities. This would help the bivalve industry to identify new market opportunities and develop specialized products in response to international demand. The high-quality bivalves produced in an environmentally sustainable manner can demand a premium in many international markets. Certification and high-quality standards can play a more important role in accessing higher-value in international markets.

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