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REVIEW

Bivalve aquaculture in warm tropical and subtropical waters with reference to sanitary water quality, monitoring and post-harvest disinfection

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Abstract *The warm water and high primary productivity of tropical estuaries allows for rapid growth and production of bivalves, but sanitary quality of molluscan shellfish poses one of the single largest impediments to development of international markets. The regulations of the US Food and Drug Administration (FDA) are outlined as representative of regulations governing international trade of fresh and frozen molluscan shellfish. The status of shellfisheries and molluscan aquaculture in several tropical and subtropical nations is presented. A number of developing nations, including Mexico, Peru and the Philippines, have established export markets for fresh scallop adductor meats because they are not considered to be a public health risk. Developing countries, including Mexico, Chile and Korea, export other bivalves by maintaining sanitary water quality in shellfish-growing areas. The inadequacy of coliform bacteria as indicators of shellfish hygiene in tropical waters necessitates research and development of alternative water quality indicators. Strategies for raising shellfish sanitary quality prior to marketing are presented, including transplant or relay, depuration and irradiation of the shucked product. Depuration is especially useful in lowering the risk of environmental, non-faecal pathogens such as* Vibrio *and can provide extra time between harvest and marketing, to allow public notification in the event of toxic algal blooms.*

Keywords: bivalves, molluscs, aquaculture, shellfisheries, shellfish sanitation, water quality indicators, depuration.

Introduction

Rationale for molluscan aquaculture

Aquaculture, by and large, has been accepted as a means of increasing fishery production in the light of decreasing natural fishery stocks. This is particularly true in a number of East Asian countries in which a relatively dense populace has traditionally relied on aquatically derived protein sources. A number of these nations have recognized the potential of mariculture of bivalve molluscs as a relatively inexpensive protein source

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for domestic use, as well as a potentially valuable source of foreign exchange earnings. Different governments, such as those representing the People's Republic of China and the Republic of Korea, have actively supported programmes to promote molluscan aquaculture.

There are a number of reasons why development of molluscan aquaculture has been considered to be highly attractive. Firstly, many of the methods for raising bivalves such as mussels, clams and oysters are well established. In many countries there is a long tradition of artisanal culture and most of the techniques are simple and readily adaptable to most parts of the world (Korringa 1976a, Quayle 1980, Angell 1986). Bivalve molluscs are filter-feeders which occupy a rather low position in the food chain. Since they depend on the natural, primary productivity of the water at the culture site there is no feeding cost (Korringa 1976b).

The warm water and high productivity of estuaries in much of the tropical developing world is another reason for the attractiveness of molluscan aquaculture development. Temperature is known to affect the growth rate of many poikilothermic (variable body temperature) animals, including bivalves (Prosser 1973). In the 5–35°C range, the rates of many physiological processes, including somatic growth, often double or triple with each 10°C increase in temperature. Thus Q_{10} effect allows for greater production of bivalves in shorter periods of time in tropical warm water estuaries. Temperate species of bivalves are known to grow faster in lower latitudes. For example, the American oyster, *Crassostrea virginica* (Gmelin), takes 3–5 years to reach half-shell market size (75–85 mm valve height, longest axis measurement) in the cool waters of Maine and Nova Scotia whereas it takes 2–3 years to reach the same size in the mid-Atlantic States and the Gulf of Mexico (Bardach *et al.* 1972). The Japanese oyster, *Crassostrea gigas* (Thunberg), experiences temperatures of 5–19°C water in Willapa Bay, Washington State (Korringa 1976a), and in the estuaries of northern Japan (Bardach *et al.* 1972). In both locations, the oysters reach marketable size in 22–24 months. In Hong Kong, the southernmost point of the natural range of *C. gigas*, the oysters are exposed to a temperature range of 11–31°C, and reach market size in 8–10 months (Mok 1973 and 1974).

There are a number of tropical oyster species which are well adapted to the warm waters of tropical estuaries and have very high growth rates. For example, the mangrove oyster *Crassostrea rhizophorae* (Guilding) takes 8–10 months to grow to half-shell market size of 65–75 mm valve height in the estuaries of Venezuela. Likewise, the oysters *Saccostrea lugubris* and *Crassostrea belcheri* in Thailand take 6–9 months to reach market size (Angell 1986). The fastest growing tropical species to be reported is *Crassostrea paraibanensis* Singaraja from Brazil. This species takes 4–6 months to grow to half-shell size of 65 mm valve height (Singaraja 1980).

The increased growth rates of temperate bivalves in warmer waters, as well as the naturally high growth rates of tropical species, leads to greater overall production of biomass per unit area and time. Raft culture of *C. gigas* in northern Japan yields 1000 $kg.ha^{-1}.year^{-1}$ of fresh shucked meat. Using the same culture techniques, the warmer waters of southern Japan produce 20 000 $kg.ha^{-1}.year^{-1}$ (Bardach *et al.*

1972). In the most productive areas of Taiwan, off-bottom culture methods produce 32 000 kg.ha^{-1}.year^{-1} of *C. gigas* shucked meats (Chen 1976). Likewise, using off-bottom techniques of bamboo frames and hanging cultch spat collectors, production of *Crassostrea iredalei* (Faustino) is in excess of 25 000 kg.ha^{-1}.year^{-1} in the Agno River estuary system in the Lingayen Gulf region of the Philippines (Rice, unpublished data, 1983). Clearly the production potential of bivalves in the warm-water estuaries of many developing nations of the tropics and subtropics is much higher than that of many industrialized nations in higher latitudes.

In terms of environmental impacts of aquaculture systems, bivalve mariculture is perceived as being one of the most benign. For example, the development of bivalve mariculture operations in estuaries and coastal waters is considered to be less destructive to the estuarine habitat than pond construction for shrimp or finfish operations (Turner 1977, Twilley 1989). Intensive cage culture of finfish has been criticized as the required feeds add to the nutrient load and lead to estuarine eutrophication (Saunders 1988, Barinaga 1990). Although bivalve mariculture may be less destructive to estuarine habitats than other aquaculture operations, it is not totally free of environmental impact. Off-bottom systems for culturing molluscs in highly productive areas are known to be responsible for increased siltation, especially where there are high levels of suspended particulates (Ottman and Sornin 1985). In addition, changes in the benthic ecology below mussel rafts have been reported. These result from increased deposition of detritus from faeces and pseudofaeces produced by the large number of mussels in a relatively confined area (Tenore *et al.* 1985, Figueras 1989). Environmental impacts of molluscan aquaculture, although significant, do not appear to be as severe as those related to pond construction or feeds required for species higher in the food chain.

A number of economic and other factors in many industrialized nations have limited the supply of molluscan shellfish, thus making the shellfish largely a luxury food. One of the most common constraints to aquaculture production in the industrialized nations is socio-political. For example, where there is a commitment to maintain the 'free-and-common' capture fishery to avoid the perceived privatization of estuary bottom by aquaculture leasing arrangements, yields are low (less than 100 kg.ha^{-1}.year^{-1} of harvestable product (Bardach *et al.* 1972). Elsewhere, aquaculture is allowed and leases are granted, but limited to on-bottom culture (Bowden 1981). Often off-bottom molluscan mariculture systems are cited as being obstructive to free navigation and unsightly. Although on-bottom molluscan mariculture is more productive than natural stock fisheries, they rarely exceed 1000 kg.ha^{-1}.year^{-1}. These yields are well below the reported production by off-bottom methods which regularly exceed 3000 kg.ha^{-1}.year^{-1} (Bardach *et al.* 1972).

A general decline of shellfisheries in many estuaries in industrialized nations has been brought about by pollution and overfishing. Oysters have been especially affected. A number of examples from the USA illustrate this. During the 1890s, oyster production in the Chesapeake Bay (Virginia and Maryland) was well over 50000 metric tonnes (mt).year^{-1}, declining in recent years to 10 000 mt.year^{-1} (Krantz 1982). Likewise, oyster production in Georgia has declined substantially since the turn of the century

from 4000 mt.year^{-1} to 15 mt.year^{-1} in 1980 (Cowman 1982). The Narragansett Bay of Rhode Island oyster production fell from 7000 mt.year^{-1} in 1910 to zero by 1957 and has not recovered (Olsen *et al.* 1980). In all of these cases, the major declines in the fisheries have coincided with human population growth and industrialization of the estuaries. In addition to pollution and overfishing, epizootic diseases such as *Haplosporidium nelsoni* (MSX) (Haskin *et al.* 1966, Ford and Haskin 1982) or *Perkinsus marinus* (Dermo) (Mackin *et al.* 1950, Andrews and Hewatt 1957, Levine 1978) have severely restricted oyster supplies. Another parasite, *Bonamia ostriae*, is also posing problems, especially among oysters of the genus *Ostrea* (Friedman *et al.* 1989).

As a result of these various factors limiting the production of molluscan shellfish in the industrialized nations, there is a considerable differential between the market prices of molluscan shellfish in many lesser-developed nations with high levels of aquaculture production, and those in Europe and North America. For example, the retail price for shucked fresh oyster meats in February 1990 was \$1.78 kg^{-1} in the Philippines (assuming \$1 = 22.5 Philippines Pesos) and \$16.50 kg^{-1} in the USA. Ideally, the price differential should be adequate incentive for entrepreneurial trade development. However, the sanitary quality of the shellfish poses one of the single largest impediments to any international trade development based on fresh or fresh-frozen molluscan fishery products (Rosario *et al.* 1982).

Requirements of countries importing molluscan shellfish

Most countries which import fresh or fresh-frozen bivalve molluscan meats have some microbiological standards which must be met. In an effort to protect public health, the USA has stringent shellfish import regulations which are administered by the US Food and Drug Administration (FDA). The first set of requirements is common to all imported food products, promulgated by the Federal Food, Drug and Cosmetic Act of 1938 as amended (Title 21, Part 101, Sections 301–392 US Code) and the Federal Fair Packaging and Labelling Act of 1912, as amended (Title 15, Sections 1451–1461 US Code). These regulations seek to assure food wholesomeness and labelling accuracy (FDA 1989a, 1989b). Imported bivalve molluscan shellfish products must conform to additional requirements of the National Shellfish Sanitation Program (NSSP) also under the jurisdiction of the FDA. The most current regulations are published by the Interstate Shellfish Sanitation Conference and are updated occasionally (ISSC 1989a, 1989b). These volumes outline requirements for water quality in shellfish growing areas, monitoring protocols, harvesting, post-harvest handling and shipping of all applicable shellfish destined for interstate trade. A separate volume (USPHS 1965) outlined the procedure for assessment of State shellfish programmes but is now obsolete and undergoing revision (S. Ratcliffe, FDA, pers. comm. 1990). All foreign countries that wish to export bivalve molluscan shellfish to the USA must initiate and negotiate a Memorandum of Understanding (MOU) with the FDA. Evidence must be presented that the exporting country has laws and procedures equivalent to the ISSC. After the MOU has been signed, the exporting country is considered as part of the ISSC and

individual certified shippers in the exporting country are listed in the FDA List of Certified Shellfish Shippers. The exporting country shellfish programme must undergo periodic review by the FDA. MOUs may occasionally be inactivated if compliance with ISSC protocols is not satisfactory. For example the MOU between England and the FDA was inactivated after gastroenteritis outbreaks in New York and New Jersey were traced to shipments of hard shell clams from England (Furfari and Chandler 1983). Presently, most countries with MOUs with the FDA are industrialized nations in the temperate zone. Lesser-developed nations with active MOUs are The Republic of Korea, Chile and Mexico. Venezuela is currently initiating the MOU negotiation process (S. Ratcliffe, FDA, pers. comm. 1990).

Overview of shellfisheries and bivalve mariculture in selected lesser-developed countries

Mexico

Mexico is geographically situated so that there are three different malacologic provinces represented: the Californian, the Panamic, and the Caribbean (Olsson 1961, Abbott and Dance 1982). Each malacologic province has specific molluscan species which occupy ecological niches. As a consequence Mexico has a relatively large number of species of bivalves it can potentially culture. Oysters which are commercially exploited include the Japanese oyster, *Crassostrea gigas*, in Baja California (Olivares 1975, Islas and Lopez 1978, Haro *et al.* 1981) and the American oyster, *Crassostrea virginica*, along the coast of the Gulf of Mexico (DeLara and Gutierrez 1977, Rogers *et al.* 1979). Tropical oysters which are harvested include *Crassostrea iridescens, C. corteziensis, C. palmula*, and *C. columbiensis* in the Pacific (Baquiero-Cardenas 1984) and *Crassostrea rhizophorae* in the Yucatan region (Littlewood 1988, Littlewood and Gordon 1988). Major species of clams and cockles include *Mercenaria campechensis, Rangia cuneata* and *Codakia orbicularis* in the Gulf of Mexico and Caribbean and *Tivela stultorum, Dosinia ponderosa, Anadara grandis, Anadara tuberculosa*, and *Chione undatella* along the Pacific coast (Baquiero-Cardenas 1984). The mussels *Mytilus edulis* and *Mytilus californianus* are harvested in Baja California.

During the last two decades, the Mexican Directorate of Aquaculture (Direccion General de Acuacultura) and the Fisheries Department (Secretaria de Pesca) has devoted considerable effort to develop molluscan aquaculture in Mexico (Baquiero-Cardenas 1984). As part of this effort, a MOU was negotiated with the US FDA which allowed for the export of oysters to the USA. The MOU at present only covers oysters produced in Baja California and the Gulf of California. Although there are considerable shellfisheries on the Mexican east coast, water quality in the growing areas is not adequate to meet ISSC requirements, precluding certification (R. Wetherell, FDA, pers. comm. 1990).

In general the adductor muscle of scallops, which rarely carries microbial pathogens, is the only portion used for human consumption. For this reason, scallops are not covered by the ISSC regulations and thus do not require FDA certification beyond those

required for non-molluscan food products. At present there are substantial scallop fisheries in Mexico, for species including *Pecten vogdesi, Argopecten circularis, Argopecten irradians amplicostatus* (Texas bay scallop), and *Argopecten gibbus* (calico scallop). The export of scallops from Mexico is reported to be 559 mt in 1987, worth 2.01 million dollars (Secretaria de Pesca 1988).

Chile

Besides Mexico, Chile is the only Latin American country with an active MOU with the US FDA. There are active shellfisheries for oysters and mussels in the southern regions of the country, well away from major population centres. Considerable effort has been expended by the Chilean government, to develop molluscan aquaculture in the region of Chiloe Island, south of the city of Valdivia. A scallop fishery exists in the north-central region of Chile in the area of Coquimbo.

The major mussel species produced in Chile are *Choromytilus chorus* with production figures of 978 mt in 1986 and 686 mt in 1987, and *Mytilus chiliensis* with production of 9005 mt in 1987. Small quantities of another mussel, *Aulacomya ater* are also produced. It has been estimated that 15–18% of the mussel production in Chile is produced by aquaculture (Gonzalo 1990). The oysters produced in Chile are the native *Tiostrea chiliensis* with production of 735 mt in 1986 and 497 mt in 1987, and the non-native *Crassostrea gigas* with production of 244 mt and 80 mt in 1986 and 1987 respectively. Aquaculture has accounted for about one third of the *T. chiliensis* production and all of the *C. gigas* production. The scallop *Argopecten (Chlamys) purpurata*, largely produced by aquaculture, yielded 258 mt in 1987. Commonly harvested clams *Mesodesma donacium* and *Protothaca thaca* are showing promise as aquaculture species as is the southern scallop *Chlamys patagonia*.

Venezuela

Since the 1970s, there has been active research in Venezuela to develop mariculture of various molluscan species. Most research has focussed on the experimental culture of the mangrove oyster *Crassostrea rhizophorae*. Total reported landings of oysters was 500 mt in 1987 (FAO 1989). Additional shellfisheries are based on the rock mussel, *Perna per* as well as venerid clams, ark clams and scallops. Statistics for 1987 (FAO 1989) show that 460 mt of rock mussels and 18 000 mt of ark clams (genus *Arca*) were landed, making Venezuela one of the largest producers of these species.

Venezuela has recently initiated the procedure for negotiating a MOU with the US FDA. Under the initial MOU, the Venezuelan government proposes to export clams harvested from the region of Isla de Margarita in the eastern region of the country (S. Ratcliffe, FDA, pers. comm. 1990). Eastern Venezuela, which is without population centres, has relatively clean waters. This area has also been the area of active aquaculture research.

Ecuador

By and large, Ecuador is more representative of most Latin American countries in terms of the exploitation of its molluscan shellfish resources. There is a small, artisanal fishery

in the country that supplies domestic needs. Examples of bivalves marketed locally are the oysters, *Crassostrea palmula* and *C. columbiensis*, the ark clam, *Barbatia reeveana*, and the swamp mussel *Mytella guyanensis*. Little has been done to augment natural harvest by aquaculture.

Potentially Ecuador could produce considerably higher yields of bivalves if simple artisanal mariculture techniques were adopted (e.g. Quayle 1980). The largest estuary of western South America, the Guayas, is Ecuador's main waterway. It is surrounded by extensive tidal flats with mangrove forest. In addition, there are minor estuaries which are environmentally similar. In the 1970s and 1980s there was a major effort to reclaim areas of mangrove swamp to construct ponds for shrimp culture, mainly *Penaeus vannemei*. Recent evidence suggests that the reclamation of swamps and excess nutrients associated with the pond drainage have led to eutrophication and a decline of natural productivity (Olsen and Arriaga 1989).

Diversification of mariculture activities, including molluscan aquaculture, has been suggested as a management option in the light of declining shrimp production (Olsen and Arriaga 1989). Sanitary water quality, especially in the Guayas Estuary, is recognized as a key impediment to full development of molluscan shellfish resources. As a result, efforts have begun to train Ecuadorian university and government personnel in the standard methods of wastewater analysis (Montaño-Armijos 1990).

Republic of Korea

With the exception of Japan, Korea is the only country in Asia to negotiate a MOU successfully with the US FDA. Although Japan has negotiated a MOU, they have not been actively exporting molluscan shellfish to the USA. In 1987, total Korean bivalve molluscan exports were 8771 mt, of which 68·5% were exported to the USA (FAO 1989, Korean Fisheries Administration Statistics). Estimated gross export earnings were $35 million. The Republic of Korea has made aquaculture a key priority and has undertaken major steps toward the maintenance of high water quality standards in shellfish growing areas (Ratcliffe and Brands 1987). In several aspects, the programmes in Korea are an excellent model for the developing world.

A number of bivalve species are produced in Korea, with a major fraction obtained from mariculture. The key oyster species is *Crassostrea gigas* with approx. 300 000 mt produced in 1987 (FAO 1989). In addition, there is a modest production of *Crassostrea rivularis* and considerable production of ark clams of the genus *Anadara*. The species produced are *A. subcrenata*, *A. granosa*, and *A. broughtoni*, with total production of 16 160 mt in 1983 (Broom 1985). Mussel production in Korea is limited to *Mytilus edulis* (synonymous with the Korean mussel *Mytilus crassitesta*) and amounted to about 40 000 mt in 1986 (Cherlamwat and Lutz 1989). Total production of freshwater molluscs, including the clam *Corbicula japonica* was 15 381 mt in 1987 (FAO 1989).

The main areas of shellfish production in Korea are in bays and estuaries along the Yellow Sea and the Korea Strait. The growing areas which are certified for the production of shellfish destined for export are in the southwestern region of the country, a predominantly agricultural area in the vicinity of Yosu City (Ratcliffe and Brands 1987). In this area, waters are monitored regularly for bacterial indicators of faecal

contamination. In addition, special studies were undertaken to determine the effects of runoff associated with rainfall on the coastal water quality. Special efforts are undertaken to limit wastes likely to degrade the quality of the growing waters. These efforts include organized cleaning activities along the shoreline, designed to remove rotting seaweed and other materials likely to promote environmental bacteria, such as *Klebsiella*, which contribute to coliform counts. The usage of agricultural manure is strictly managed in the coastal areas, including a prohibition of its usage directly adjacent to the shellfish growing areas. There is an active programme for the upgrading of toilet facilities in homes in the nearshore areas. Dry toilets similar to those outlined by Dufour *et al.* (1985) are in use. Nightsoil is collected and transported to specialized facilities where it is composted well away from shellfish growing areas.

China

China, considered to be the cradle of aquaculture dating back to 475 BC (Bardach *et al.* 1972), may well be the world's largest producer of bivalves. Three species of mussels are produced: *Mytilus edulis*, in Shandong Province and north to the Yellow Sea; *Mytilus coruscus*, mostly in the East China Sea; and *Perna viridis*, south from Fujian Province (Zhang 1984). Mussels account for 151 768 mt (12·3%) of China's shellfish production, the largest in Asia (FAO 1989). Although mussel production is rather high, production is considered insufficient to meet domestic market demands. For this reason, hatcheries produce mussel spat in the Yellow Sea region to augment natural spatfall (Zhang 1984). The major growing area for oysters in China is in Guangdong (Canton) Province. The major species produced, *Crassostrea gigas, C. rivularis* and *Saccostrea cucullata*, amounted to well over 150 000 mt in 1987. Likewise, the production of various species of clams and scallops was estimated to be 889 000 mt in 1987. A total of 45 680 mt of freshwater molluscs, such as *Corbicula fluminea* and *C. japonica*, were landed (FAO 1989). This impressive production (1·24 million tonnes) has been used solely for domestic consumption.

Taiwan

The market economy of Taiwan has allowed export marketing of mariculture products. Much like Ecuador, Taiwan has concentrated on the production of pond-reared shrimp as a major export commodity. Unlike Ecuador, Taiwan has a long tradition of aquaculture which is highly diversified. Key species produced are the Japanese oyster, *Crassostrea gigas*; the poker chip venus, *Meretrix lusoria*; the Manila clam, *Tapes philippinarum*; the blood cockle, *Anadara granosa*; and the Asian freshwater clam, *Corbicula fluminea* (Chen 1976).

Water temperature in Taiwan estuaries ranges from 15 to 28°C and supports high growth rates and productivity among molluscs. In Taiwan, average production of oyster beds, using off-bottom techniques, is 1·5 $mt.ha^{-1}.year^{-1}$. The most productive areas produce about 32 $mt.ha^{-1}.year^{-1}$. Total production of molluscs by Taiwan was in excess of 19 000 $mt.year^{-1}$ in 1975 (Chen 1976), the majority of which have been marketed domestically. Taiwan has the potential for expanding production by adding to

production areas, but sanitary water quality of shellfish growing waters has been the major impediment to development.

Philippines

Much like Taiwan, aquaculture in the Philippines is highly diversified in terms of species and culture techniques. The commercial production of molluscs in the Philippines is reviewed by Talavera and Faustino (1933) and Edwards (1981). Among the key bivalves is the mussel *Perna viridis*, with production of approx. 18 000 mt in 1986 (Cherlamwat and Lutz 1989). The oysters produced are *Crassostrea iredalei, C. malabonensis, Saccostrea cucullata*, and *S. echinata* (Carreon 1969). Total oyster production was estimated to be 16 000 mt in 1987 (FAO 1989). There is a small commercial scallop fishery, with the moon scallop, *Amusium pleuronectes*, as a key target species (del Norte *et al.* 1988). Scallop landings amounted to 529 mt in 1987 (FAO 1989). These scallops form the basis of an export market, with Japan as the major customer.

There have been attempts by Philippine trading companies to develop sustained export markets for shucked and frozen molluscan shellfish other than scallops (Rosario *et al.* 1982). Sanitary water quality of growing areas has been one of the main reasons for the failure of such attempts (Rice and Poquiz 1983). The major bivalve mariculture areas are near to the largest and most populated island of Luzon. The largest production area of oysters and mussels is located in Manila Bay near the city of Cavite. The other large production area (mainly oysters) is in the Agno River estuary system in and around Dagupan City. Both the Manila Bay and the Agno River estuary system are in highly urbanized areas. Recent data on water quality in Manila Bay is lacking, but a study by Maaliw *et al.* (1989a) has shown that total coliform bacteria regularly exceed 2400 MPN.100 ml^{-1} in the vicinity of the oyster grounds in the Dagupan–Agno River Estuary. Another study conducted in the same area shows lowered dissolved oxygen and increased suspended solids in the water, consistent with domestic sewage loading (Maaliw *et al.* 1989b).

Thailand

Thailand is another southeast Asian country that produces a considerable harvest of bivalve molluscs. Estimates of total bivalve production exceed 300 000 $mt.year^{-1}$. Thailand is the largest producer of the mussel *Perna viridis*, and is the second largest producer of all mussel species in Asia, after China (Cherlamwat and Lutz 1989). The major production areas of mussels are located in the upper Gulf of Thailand (Vakily *et al.* 1988). Oysters produced include *Crassostrea lugubris, C. belcheri, Saccostrea cucullata*, and *S. commercialis* (Amornjaruchit 1988). The major oyster culture provinces are Chon Buri, Rayong and Chanthaburi in the northern Gulf of Thailand and Surat Thani, Chumphon, Songkhla and Patthani on the Gulf of Thailand coast of the Malay Peninsula (Brohmanonda *et al.* 1988). Among the various species of clams in Thailand, the most commonly harvested is *Paphia undulata*, with production of 85 500 mt in 1987 (FAO 1989). In addition, there is considerable production of the ark clam *Anadara granosa* (Tookwinas 1983). Approx. 900 mt of *A. granosa* are produced annually, which

is insufficient to meet domestic demand. As a result, approx. 80% of the market for ark clams in Thailand is imported from Malaysia.

In order to reduce this importation of ark clams, there is an active programme to introduce anadarid culture in the Songkhla Province near the Malaysian Border (P. Chittapong, Prince of Songkhla Univ., pers. comm. 1988). In another project, oyster culture is being promoted in Ranong Province on the Andaman Sea, near the Burma border (Madhu 1990). Active oyster growing areas were estimated to be 7064 ha in 1983. That same year, areas with potential as oyster mariculture sites were estimated to be 35 933 ha (Brohmanonda *et al.* 1988). This suggests that production could be increased substantially.

In concert with the Thai policy of promoting mariculture, there is concern about water quality in shellfish growing areas. A survey of heavy metals showed no problem with elevated concentrations in key species of bivalves from any of the shellfish-growing areas (Phillips and Muttarasin 1985). The agency responsible for monitoring water quality, The Office of the National Environment Board (ONEB), uses coliform bacterial standards (M. Tabucanon, ONEB, pers. comm. 1990). Data suggest that the major shellfish-growing areas in the upper Gulf of Thailand have regularly high coliform counts, well above ISSC standards (Musig and Ruttanagosrigit 1983, Saitanu *et al.* 1987).

Indian subcontinent

There are a number of species of bivalves in the southern Asia region that are exploited by the artisans. In India these include the oysters *Crassostrea gryphoides* (Durve and Bal 1962, Durve 1965) and *Crassostrea madrasensis* (Stephen 1980). Oysters native to Pakistan include *Crassostrea arakensis, C. madrasensis, Saccostrea glomerata*, and *S. cucullata* (Asif 1979). At present, Pakistan is interested in developing many underutilized shellfish resources, including oysters and venerid clams of the genus *Marcia* (I. Zehra, Univ. of Karachi, pers. comm. 1990). In Sri Lanka, there has been experimental culture of the mangrove oyster *Saccostrea cucullata* (Pinto and Wignaraja 1980).

Africa

Throughout Africa, naturally occurring stocks of molluscs are exploited artisanally. The Indo-Pacific mangrove oyster *Saccostrea cucullata* is harvested in the East African countries of Madagascar (Rabesandratana 1971) and Tanzania (Bwathondi 1982). A few African countries have initiated projects to experiment with bivalve mariculture. Experimental culture of *Crassostrea gasar* has been carried out in Nigeria (Bardach *et al.* 1972, Ajana 1979, 1980). In Sierra Leone, efforts have been made to culture *Crassostrea tulipa* (Kamara and McNeill 1976, Kamara 1982). The Mediterranean mussel *Mytilus galloprovincialis* and the Japanese oyster *Crassostrea gigas* are being cultured in Algeria (Zemmouri 1990). It is likely that introduction of small-scale bivalve aquaculture would result in increased production in most of Africa, but in order to make this economically viable the markets would have to be developed.

Microbiological indicators of water quality

There are a considerable number of human diseases which are transmitted via faecal contamination of water. Table 1 lists a few of these diseases, the disease agents and some of their symptoms. Many of these diseases, especially the protozoan and helminthic parasites, are much more common in the tropics. Although many of these diseases are very common, they are not always present in faecally contaminated waters. Furthermore, even when they are present, the absolute numbers of the viable and active pathogens may be very small, making detection extremely difficult. These factors make

Table 1. Pathogens found in human excreta (modified after Vaughn and Landry 1984, Piedrahita and Tchobanoglous 1987)

Biological group	Organism	Disease
Viruses		
	Adenovirus	respiratory infections and gastroenteritis
	Coxsackievirus	various
	Echovirus	various
	Hepatitis A virus	infectious hepatitis
	Norwalk virus	gastroenteritis
	Parvovirus-like	gastroenteritis
	Poliovirus	poliomyelitis
	Reovirus	various
	Rotavirus	gastroenteritis
Bacteria		
	Camphylobacter	gastroenteritis
	Pathogenic *Escherichia coli*	gastroenteritis
	Pathogenic *Klebsiella* sp.	respiratory infections
	Salmonella typhi	typhoid fever
	other Salmonellae	food poisoning
	Shigella spp.	bacillary dysentery
	Vibrio cholerae	cholera
	Vibrio vulnificus	septicaemias
	other vibrios	diarrhoea
	Yersinia spp.	yersiniasis
Protozoa		
	Balantidium coli	diarrhoea
	Entamoeba histolytica	amoebiasis
	Giardia lamblia	giardiasis
	Isospora spp.	intestinal parasitism
Helminthic parasites		
	Ascaris lumbricoides	ascariasis
	Clonorchis sinensis	clonorchiasis
	Diphyllobothrium latum	diphyllobothriasis
	Fasciolopsis buski	fasciolopsiasis
	Schistosoma spp.	schistosomiasis
	Paragonimus westermani	paragonimiasis

programmes for the direct detection of pathogens in natural waters expensive and potentially ineffective because of the low probability of detection of all potential pathogens. For this reason, microbial indicator organisms of faecal contamination are favoured.

Ideal microbiological indicators would have the following characteristics:

1. they should be present in very high numbers in human faeces;
2. they should be present consistently in human faeces;
3. the persistence or survival of the microbiological indicator in natural waters should approx. match the survival of pathogens. The indicator dying off before the pathogens might lead to public health problems. The indicator persisting much longer than the pathogens might raise concerns of risk when none is present;
4. the techniques for detection and enumerating the indicator should be inexpensive, reliable, and simple.

Most industrialized countries of the temperate regions have adopted either total or faecal coliform bacteria as the indicators of faecal contamination. Total coliform bacteria are gram-negative bacilli which ferment lactose. Included in this group are the genera *Escherichia, Enterobacter, Klebsiella,* and *Citrobacter.* Faecal coliforms or thermotolerant coliforms are a subset of the total coliform group, *Escherichia* and *Klebsiella,* which are capable of fermenting lactose at elevated temperatures (44°C) (Greenberg and Hunt 1985). For the most part, the coliforms fulfil the above stated suitability requirements for microbial indicators, at least for some bacterial diseases. Indeed, the major decline since the 1920s of shellfish-borne bacterial disease, such as typhoid fever, has been attributed in part to water quality monitoring based on coliform indicators (Richards 1975, Cabelli 1986).

Total or faecal coliform indicators are the basis for water quality classification in shellfish-growing areas under international MOUs with the US FDA (ISSC 1989a). For example, coliform-based water quality standards are applied in the Australian State of Tasmania (Ratcliffe and Lucas 1982), New Zealand and Canada, all with MOUs. As stated previously, most of the countries with MOUs are developed countries in the temperate zones. A number of developing countries in the tropics, including Thailand are adopting coliform water quality standards. In Ecuador, total and faecal coliform indicators are used to assess water quality of public water supplies (Solorzano 1989) as well as coastal and estuarine waters (Montaño *et al.* 1990).

In spite of widespread use in developed countries in the temperate zone and adoption by some countries in the tropics, there is a growing body of evidence that suggests that coliform indicators may not be appropriate for tropical waters. A number of studies have shown poor correlation between bacterial pathogens and coliform indicators. For example, a study in Mangalore, India showed very poor correlation between pathogenic *Vibrio cholerae* and faecal coliform indicators. In some samples of water and shellfish, faecal indicators were relatively low and *V. cholerae* was present, and in other samples *V. cholerae* was absent although faecal indicators were high (Mathew *et al.* 1988). In another study, one of the key faecal coliform bacteria, *Escherichia coli,* has been shown

to have very high survivability in tropical environments, especially in waters with organic enrichment, such as effluents of rum distilleries (Fuentes *et al.* 1983, Santiago-Mercado and Hazen 1987). The high survivability of *E. coli* in tropical environments is such that it has been found in areas of Hawaii and Puerto Rico which have not had recent faecal contamination (Fujioka *et al.* 1981, Carillo *et al.* 1985). Another study showed that the survival of *E. coli* can greatly exceed the survival of other pathogens (Lopez-Torrez *et al.* 1987). These findings have led to the suggestion that coliform indicators would be unacceptably conservative in their estimation of human health risks from faecally transmitted pathogens (Hazen *et al.* 1987).

Several other studies have shown that bacterial species, other than *E. coli*, are often present in high numbers in warm tropical or subtropical waters. For example, work in Louisiana showed that *E. coli* are the dominant faecal coliform species during the cooler months (below 21°C), but non-*E. coli* faecal coliforms, especially environmental isolates of *Klebsiella pneumoniae*, predominate during the warmer months (>21°C) of May–September (Paille *et al.* 1987). The warm temperatures favoured the growth and survival of *Klebsiella* with *Escherichia* populations remaining fairly constant. The *Klebsiella* often outnumbered *E. coli* 1000:1 during the warm months. In cases of this type, where environmental-source bacteria are greatly outnumbering faeces-associated bacteria, direct assessment of *E. coli* is suggested as a more accurate indicator (Kilgen *et al.* 1988). The suggested methodologies include plate counts with 24-hour indole and citrate tests (Anderson and Baird-Parker 1975) or the more sensitive fluorogenic assay using lauryl sulphate tryptose (LST) and methylumbelliferone glucuronide (EC-MUG) (Feng and Hartmann 1982).

In at least one tropical country, policy makers have recognized that background numbers of coliform bacteria in water are normally high. For this reason, the National Pollution Control Commission of the Philippines has set 1000 MPN.100 ml^{-1} total coliform as the level most likely to indicate recent faecal contamination (NPPC 1978). In contrast, a 70 MPN.100 ml^{-1} total coliform water quality standard is required by the US FDA under MOUs (ISSC 1989a).

Bacteria of the genus *Vibrio* are another concern in warm tropical or subtropical waters (see Colwell 1984 for a review). Many of the vibrios present in the environment are pathogenic and are not necessarily associated with human faeces. Thus, they are not predicted by the presence of coliform indicators. Of health concern are *V. parahaemolyticus, V. cholerae*, and *V. vulnificus*, which cause a number of diseases ranging from mild gastroenteritis to cholera and septicaemias. Vibrios have been found in shellfish and growing waters in Louisiana during the warm summer months (Kilgen *et al.* 1988) and in Thailand (Paw *et al.* 1988).

In addition to the evidence that coliform indicators in warm waters may be inadequate to predict bacterial pathogens, there is also evidence that they lack the ability to predict risk from some viral pathogens. Portnoy *et al.* (1975) reported an outbreak of viral hepatitis in Louisiana traced to waters certified using coliform standards. Other outbreaks of viral diseases, including Norwalk virus-associated gastroenteritis, have been traced to shellfish from waters presumed to be free from faecal contamination

based on coliform indicators (Gerba *et al.* 1979, Ellender *et al.* 1980, Richards 1985, Cole *et al.* 1986).

Proposed alternative indicator systems

Due to the demonstrated inability of coliform indicators to serve as an index of health risk from enteric viruses and warm-water vibrios, it has been suggested that a nationwide, multidisciplinary study should be conducted in the US to develop alternatives to the standard coliform indicators (Kilgen *et al.* 1988). Due to the increased survival of *E. coli* in tropical waters, the high incidences of *Klebsiella* and vibrios, as well as the greater incidences of waterborne protozoan and helminthic parasite diseases, alternative indicators are particularly necessary. The simplest alternative indicator to total and faecal coliforms, as previously discussed, would be the enumeration of *E. coli* in water and shellfish. This would eliminate the problem of positive faecal coliform readings due to environmental *Klebsiella* (Kilgen *et al.* 1988). Taking this approach one step further, it has been suggested that assays for human-specific *E. coli* strains could differentiate between human and lower animal wastes. Alternative indicator bacteria have been suggested as a means of maintaining an inexpensive and simple faecal indicator system with a minimum of interfering environmental strains. Alternative bacterial indicators include faecal streptococci (Cohen and Shuval 1972) and *Clostridium perfringens* (Bisson and Cabelli 1979, 1980, Madden *et al.* 1986).

Most countries with well-developed economies use sewage treatment facilities in major population centres. Sewage treatment facilities are known to eliminate bacterial pathogens and bacterial indicators effectively; but some viruses pass through commonly used final chlorination steps and maintain viability (Snead *et al.* 1980, Keswick *et al.* 1985, Cabelli 1989a, 1989b). The need for viral indicators in such instances has been recognized. Richards (1985) suggested the use of poliovirus as an indicator. This would be useful in countries in which children are regularly vaccinated with attenuated virus. Bacteriophages have been suggested as potential viral indicators (Vaughn and Metcalf 1975, Cabelli 1989a, 1989b). Bacteriophages as sewage indicators have the advantage of being present in high numbers in most treated sewage. Bacteriophage assays are usually simple to perform and are relatively inexpensive.

Another approach which has been suggested is to use direct molecular probes using complementary DNA (cDNA) strands as an assay tool. The cDNA probes could be used to demonstrate unambiguously the presence of DNA sequences in specific pathogens or selected indicators (Jiang *et al.* 1986, Margolin *et al.* 1987). There are problems associated with this type of molecular approach, one of which is a high price tag, because of the necessity for very specialized equipment and personnel. Another problem is that there is no differentiation in the assay between live or viable pathogens and non-viable DNA fragments. Molecular approaches of this type, if adopted in developing countries (and developed countries as well), would be most appropriate for occasional confirmatory studies to supplement other, simpler, methods. Currently, a chemical assay for coprostanol, a compound derived from cholesterol and found in mammalian faeces, is being used in Thailand as a confirmatory method in conjunction

with standard coliform indicators. This assay, although useful, is considered by Thai investigators to be overly complex and expensive for routine use (M. Tabucanon, ONEB, pers. comm. 1990).

Post-harvest strategies for disinfection of shellfish

In addition to giving careful attention to the maintenance of sanitary water quality in shellfish-growing waters, various methods are used to cleanse, or otherwise rid shellfish of potential pathogens. The more common methods include relaying and depuration. Relaying involves moving shellfish from moderately polluted waters to other waters known to be relatively free from contaminants and allowing sufficient time (usually several weeks or months) for purging of potential pathogens (Supan and Cake 1982). Depuration involves keeping shellfish in purified seawater at a shore-based facility for a short time (usually 48 hours) which is sufficient to purge most potential pathogens (Furfari 1966). It has been recently suggested that the use of ionizing radiation may be a viable means for assuring safe, pathogen-free fresh shucked oysters and other shellfish (Kilgen *et al.* 1988).

Depuration is one of the most commonly used means of disinfecting shellfish. Since the turn of the century, depuration has been recognized as a means of ridding bacterial pathogens from guts of oysters (Herdman and Bruce 1899). More recently, depuration has been suggested as a means of cleansing tropical oysters (Rosario *et al.* 1982). For depuration in tanks to be effective, care must be taken to ensure that water flow is sufficiently high to maintain adequate dissolved oxygen levels and that temperature, salinity and stocking densities are maintained at proper levels (Furfari 1966). Well-managed depuration systems have been shown to eliminate coliform bacteria from *Crassostrea iredalei* within 48 hours (Gacutan *et al.* 1986, Palpal-latoc *et al.* 1986). A number of pathogenic bacteria, including *Shigella flexneri, Salmonella typhimurium,* and *Franciscella tularensis* are depurated at the same rate as coliform indicators (Janssen 1973). Because of its effectiveness in eliminating bacterial pathogens, in a number of countries depuration has become an industry standard. For example, depuration is required in the mussel-producing Galicia region of Spain (Ledo *et al.* 1983). Commercial depuration of Sydney rock oysters, *Saccostrea commercialis*, is a widespread practice in New South Wales, Australia (Souness *et al.* 1979, Thison and Fleet 1980). Chile is now starting depuration to support exportation of shellfish under their MOU with the US FDA (L. Chandler, FDA, pers. comm. 1990).

In spite of its acceptance in many countries, depuration alone, without attention to water quality in the growing areas, is not sufficient to assure safe shellfish (see reviews by Richards 1988, Canzonier 1988). Firstly, depuration of shellfish with very high bacterial loads is not commercially feasible because of the excessively long times required for complete purging (Heffernan and Cabelli 1971). For example, shipments of commercially depurated and fresh-frozen oysters *Crassostrea iredalei* were rejected on entry to Singapore after being grown in waters later shown to have a very high total coliform levels (Rice and Poquiz 1983, Maaliw *et al.* 1989a).

There is evidence that 48-hour depuration may be insufficient to purge all viral pathogens. Gastroenteritis outbreaks, possibly Norwalk virus-associated, have been traced to depurated hard clams *Mercenaria mercenaria* (Furfari and Chandler 1983) and oysters (Gill *et al.* 1983). Gastroenteritis has been attributed to Norwalk virus from depurated Sydney rock oysters (Murphy *et al.* 1979).

In addition to the epidemiological data, there is a growing body of direct evidence suggesting that viruses are not depurated as rapidly as bacteria. The coliphage S-13 virus takes between several days and weeks to be fully depurated under optimum conditions (Canzonier 1971). Poliovirus in oysters has been shown to depurate fully within 3 days at 17°C with 1·8% salinity; but hepatitis A was persistent beyond 5 days, an average of 58% of the initial viral load remaining at that time (Sobsey *et al.* 1987). In another study which compared rates of bacterial and viral depuration, cricket picornavirus, used as a viral tracer in individual oysters, showed that the elimination of the virus was highly variable ranging from zero to 2 log reduction in a 10-day period; but coliforms were fully eliminated in 48 hours (Scotti *et al.* 1973). Current work with the MS-2 male-specific (F+) coliphage indicates a very high retention of viable virus particles after weeks of depuration (Burkhardt and Rippey, FDA, pers. comm. 1990).

Toxic algal blooms

Other threats to human health associated with bivalves are the various toxins associated with some species of dinoflagellates and toxic phytoplankton. The problem is recognized worldwide. In some areas, monitoring programmes may indicate the necessity to cease harvest of shellfish beds for up to several months (see reviews by Anderson *et al.* 1985, Anderson 1989, Shumway 1989). Such closures are potentially catastrophic for local shellfish industries. Toxic algal blooms in a number of countries, including Tasmania, Taiwan, Guatemala, Korea, Hong Kong and Venezuela, have resulted in closures of shellfish beds (Anderson 1989). In the Philippines, toxic algal blooms have been responsible for cessation of virtually all molluscan shellfish exports and bans on shellfishing for local consumption (C. C. Baylon, Univ. of Philippines, Visayas, pers. comm. 1990).

There is some evidence that toxins can be depurated from shellfish by treating the seawater with ozone (Gacutan *et al.* 1984, Blogoslawski 1988). The bivalves need to be actively pumping ozonated water for the depuration of algal toxins to be effective. Since the various algal toxins are toxic in very small (nanogram or picogram) quantities, a single animal failing to pump water actively can be a health threat. Thus, depuration of algal toxins is risky in large-scale commercial or other applications where individual shellfish are not monitored.

Conclusions and recommendations

Of critical importance to the sanitary quality of shellfish is the cleanliness of source waters. A number of developing countries have been able to establish mariculture

operations in sparsely populated areas with good water quality. Examples of these countries are Mexico, with oyster farms in the Baja California region; Chile, with considerable mariculture in the southern regions; and Venezuela, with mariculture development well away from the population centres.

Most developing countries, however, do not have the option of developing shellfish resources in lesser populated areas, because optimum shellfish growing areas may be in coastal areas or estuaries which are urbanized or sustain moderate density, rural populations. In these instances, shellfish production is not precluded, but extra efforts must be undertaken to assure sanitary quality of the harvested product. The first step is to assess shellfish-growing waters, using the best available microbial indicator system. This assessment would include the classification of waters and identification of areas with lower pollution levels which may allow for relay of stock. To make these baseline studies possible, adequately trained technical personnel are necessary, as well as adequate laboratory facilities in close proximity to the growing areas. After growing areas are established and there is a programme of ongoing water quality monitoring, a strong commitment must be made to the management of sewage and faecal wastes.

Korea, with oyster grounds adjacent to small family farms, is a good example in this respect (Ratcliffe and Brands 1987). Adequate dry toilet facilities are maintained in each house. Nightsoil is collected and composted in areas well away from shellfish beds, and there are restrictions on the use of manures and nightsoil in proximity to the shellfish beds.

There is strong evidence that coliform indicator standards, although used widely in temperate waters, are much less reliable in the tropics. Reliance on coliform standards for water quality may overestimate potential health risks, thus developing nations in the tropics may be unduly burdened in their efforts to develop their molluscan shellfish resources. Efforts are necessary to develop microbial indicators which are more appropriate for the tropics. Alternative indicators must be rapid, simple and inexpensive to be of use as a monitoring tool.

In addition to efforts to maintain water quality in growing areas, depuration may be necessary as an added safety measure. Depuration in warm-water areas would also be useful in lowering periodically occurring non-faecal bacterial pathogens, such as *Vibrio*. Depuration as a general practice would also allow time (2–3 days) between harvest and marketing of bivalves. This extra time period could allow for harvest restrictions and warnings to the public by health officials in the event of sudden toxic algal blooms appearing.

Finally, although efforts to assure water quality in shellfish growing areas may be in progress, export markets for bivalves might be established based upon the sale of fresh or frozen scallop adductor muscle meats. Many species of scallops are harvested in deep waters, well away from contaminant sources. In addition, water-borne pathogens are not known to accumulate in the muscle tissues, the most commonly consumed portion of the scallop. Active exporters of scallop adductor muscle meats are Mexico and Chile who have very successfully developed markets, whilst working toward clean growing waters for other bivalve species.

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