

PART 2

PEARL OYSTER HEALTH MANAGEMENT

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2.1 Introduction

The pearl production industry has evolved significantly since its first development in Japan at the turn of the century. Expansion volume and species cultured for pearl production, principally throughout the Asia-Pacific region, has increased attention to health management, since pearl production relies entirely upon the health of the oyster. The pearl is a product of a strong immune response to soft-tissue irritation. The stronger the immune system, the better the pearl quality. However, in order to produce cultured pearls, the mother-of-pearl oyster (MOPs) receives regular handling, including tissue surgery to introduce the irritant ("nucleus") for the cultured pearl. Although pearl oyster aquaculture has not faced the types of disease epizootics which have impacted edible molluscs elsewhere in the world, the ongoing development of the industry necessitates movements of oysters, equipment and people that warrants increased attention to the risk of disease introduction and spread, and awareness of health management measures that can reduce or prevent such risks.

2.1.1 Purpose, approach and target audience

The purpose of this manual is to provide technical guidance in managing the health of pearl oysters, based on a review of the literature of South Sea pearl oysters. It is, however, hoped that the procedures outlined in this manual will be equally useful for health management of other pearl oyster species.

The first section deals with general information related to husbandry and handling, hatchery production; the second concerns introduction and transfers and risk assessment; the third provides detailed protocols for disease diagnostics; the fourth deals with disease zoning; the fifth deals with disease outbreak scenarios; the sixth describes the development of national strategies on aquatic animal health; and the last section provides useful references.

This manual is intended for people at national and state agencies or institutes and private sector individuals involved in pearl oyster health management both at farm and hatchery production levels.

2.2 GENERAL

All commercially important species belong to the bivalve Family Pteriidae (Gray, 1847), a sister Family to the true oysters, the Ostreidae (Rafinesque, 1815). All species discussed in this manual fall in the genera *Pinctada* (Röding, 1798), the pearly oysters; or *Pteria* (Scopoli, 1777), the winged oysters.

2.2.1 Husbandry and handling

Introduction

Tropical and sub-tropical sub-tidal bivalve species, such as *Pinctada* and *Pteria*, do not adapt as readily as inter-tidal or temperate species to rapid changes in temperature, salinity, turbidity and water pressure. Thus, rapid environmental changes can induce significant physiological stress. Such stress can reduce resistance to disease and infection by opportunistic pathogens (Snieszko, 1974), thus, this factor is a key consideration for all the husbandry and handling techniques recommended below for pearl oyster health management.

Collection

Movement of adult pearl oysters from deep-water sources, for transfer to holding tanks/nets/cages in shallow water, should take into account changes in water pressure

and temperature, where possible. Any extreme environmental changes should be followed by a period of convalescence with minimal/no handling, prior to further transportation (Dybdahl and Pass, 1985; Pass, Dybdahl and Mannion, 1987; Dybdahl, Harders and Nicholson, 1990). The period required will vary depending on the degree of environmental change, pearl oyster size, species, and level of shell fouling (epibionts). Where such information is not known, it is recommended that sub-samples of oysters from different species or size-groups be held in hanging baskets at the collection site for varying periods prior to transfer to the farm site. This will provide collection site-specific information required to determine the optimum convalescence period needed to reduce mortalities. Convalescence periods may range from 24 hours to 1 week.

Spat collection also requires care, although depth and temperature considerations are less important as larval oysters tend to frequent the upper water column prior to settlement (Monteforte, Kappelmann-Pina and Lopez-Espinosa, 1995). Once collected in spat collector bags, the first health management measure is to minimise unwanted hitch-hikers, such as spat of other bivalve species, predators and fouling organisms. This is necessary to reduce food competition and/or asphyxiation. Such stresses during early development may compromise the quality of the shell and oyster health later in life. Removal of spat from the collector bags also requires care. Air exposure gives good detachment results but is particularly stressful to this stage of development of sub-tidal species and was found to have inferior post-detachment results compared with trials using hypersaline water (40–45 ppt) or sub-ambient salinities (25–30 ppt) (Taylor, Rose and Southgate, 1997a). Re-attachment and survival was found to be 100 percent 24 hours post-detachment using saline treatments.

Handling

Handling for monitoring, sorting, defouling or transfer purposes should be minimised as much as possible and undertaken under shaded conditions or where the pearl oysters can easily be immersed or kept wet with ambient seawater. Transportation requires specialized equipment to ensure adequate water exchange, maintenance of ambient water temperatures and to avoid overcrowding and particulate contamination (Pass, Dybdahl and Mannion, 1987; Dybdahl, Harders and Nicholson, 1990; Joll, 1994; Norton, 1994). No handling is recommended during convalescence periods or during seasons when water temperatures favour proliferation of infectious microbes or toxic algae. Handling stress, in addition to defence against opportunistic infections, is likely to accelerate pearl oyster health problems.

Defouling

Fouling organisms, also known as epibionts, affect pearl oysters, and other bivalves they use as substrate, in a number of different ways. Encrusting coralline colonies and sponges can spread over the hinge or shell margins inhibiting normal opening and closure for feeding and respiration. Heavy fouling may also increase the amount of mechanical energy required for shell opening. Widman and Rhodes (1991) noted a possible correlation between broken ligaments and barnacle colonization of the shells of bay scallops, *Argopecten irradians* during a growth study. Shell edge encroachment can stimulate mantle retraction and this, in turn, can cause permanent shell deformities (“double-back” Taylor, Southgate and Rose, 1997). Excessive colonization also significantly increases the weight of suspended cages or lines, to the extent that the line may sink in the water column or the oysters get stripped-off. If suspended over an unfavourable bottom, this can further reduce the oysters chances of survival. Other direct impacts of fouling can be competition for particulate nutrients (Lesser *et al.*, 1992), e.g. with filter-feeding organisms such as spionid polychaetes, barnacles, sponges and corals; and mechanical blockage of water circulation through holding cage mesh (Parsons and Dadswell, 1992). Interestingly, Lodeiros and Himmelman (1996) found

that growth of the tropical scallop, *Euvola ziczac*, was more severely inhibited by fouling of the pearl nets than by fouling directly on the shells, although, heavily fouled shells demonstrated higher mortalities than those with little surface colonisation. Thus, pearl oysters grown on long-lines, which are known to be heavily fouled, may not require as much cleaning as pearl shells held in suspension cages.

Fouling is usually controlled by manual removal (machete, blunt chisel, high pressure water hose) with frequency of cleaning varying with the nature of the fouling community, grow-out technology, holding depths and season of proliferation. Inevitably this increases the amount of handling required (see Section 2.2.1) and care is required to minimize the subsequent stress on the oyster. Ideally, methods which minimize removal from the water will reduce the stress of handling, e.g. underwater defouling by divers or cleaning of cages or individual oysters in tanks with flow-through seawater. Unfortunately, this stripping activity also means that the fouling organisms remain immersed and this increases their chances of survival, proliferation and re-attachment. This can be circumvented by moving the cages to a remote defouling station for either immersion or demersion cleaning. Interestingly, *P. maxima* appears tolerant of defouling, with maximum growth being demonstrated in oysters cleaned most frequently (every 2 or 4 weeks) (Taylor, Southgate and Rose, 1997). This is in contrast to other species, where defouling is correlated to reduced growth rates (Parsons and Dadswell, 1992).

As with other suspension-grown bivalves, holding depth may affect the degree and rate of fouling, with reduced fouling at greater depths (MacDonald and Bourne, 1989; Côté *et al.*, 1993; Claereboudt *et al.*, 1994; Lodeiros and Himmelman, 1996). Studies of growth of the winged oyster *P. penguin*, found mortalities of uncleared oysters decreasing with increasing depth from 40 percent at the surface, to 33.3 percent at 4 m to 6.7 percent at 8 m in 10 m deep water (Smitasiri, Kajiwat and Tantichodok, 1994). Chlorophyll α concentrations did not appear to vary with depth. In deeper water grow-out sites, however, the effect of surface fouling and cleaning has to be weighed against the decrease in food availability with increasing depth (MacDonald and Bourne, 1989; Côté *et al.*, 1993; Claereboudt *et al.*, 1994; Lodeiros and Himmelman, 1996).

The use of antifouling paint (Lee, 1992) or antifouling wax (Dybdahl, Harders and Nicholson, 1990) has proven useful in reducing the concentration and rate of colonization of both holding cages and shell by fouling organisms. However, the composition of any anti-fouling agent must be carefully assessed, since many are designed to combat mollusc settlement and may be toxic to the pearl oyster (especially larval and seed stages). The effect of any chemical on the ecology and water quality of the grow-out site also needs careful assessment. A rich fouling community usually indicates a healthy aquatic environment. An advantage of non-toxic anti-fouling agents, however, is the reduced amount of handling required.

Surgery

The most obvious disease concern with respect to surgery is the opening of the soft tissue which forms the first physical defence against tissue infection. In addition, the nucleus stimulates a defence response that is energetically costly to the pearl oyster. If not in optimum health, this tissue trauma and defence response may weaken the oyster to the extent that it may cease feeding and die. The haemocyte-mediated response to the artificial nucleus can also divert defence resources away from other irritants or infections, rendering the oyster more susceptible to opportunistic infections. Thus, a post-surgery convalescence period is recommended (as with post-collection and post-transportation).

Physiological stress induced by prying open the shell, holding in open air, etc., can be reduced by using relaxants (Norton, 1994). In addition, pre-operation treatments, such as varying seawater flow or feed to inhibit or stimulate gonad development, increase

physiological stress. Although high survival rates (mean of 75.3 percent) are reported in *P. martensi* (Deng *et al.*, 1995), special care is required to prevent exposure to additional stresses. Much lower survival rates and nucleus-retention are reported in *P. nigra*, depending on the development stage of the gonad. The highest survival (62 percent) and nucleus retention (61.5 percent) occurred as the gonads were enlarging (Meng *et al.*, 1994). This period coincides with the use of energy resources for gametogenesis, a process which is curtailed in conditions of energy deficit (MacDonald and Thompson, 1986; MacDonald, Thompson and Bayne, 1987; MacDonald, Thompson and Bourne, 1991; Thompson and MacDonald, 1990). This means that energy is available to repair tissue damage due to surgery as well as additional physiological challenges. Post-spawning oysters ("shrinking" and "transparent" stages) would have the least energy reserves and the resting stage of gametogenesis usually coincides with somatic growth, which may or may not reflect energy availability for use in tissue defence.

The different types of graft tissue used may also play a role in pearl oyster health and pearl formation (Tun, 1994; Wada, 1996; Wada and Komaru, 1996). Autograft methodology, using tissues from the individual being seeded, is the least likely to provoke an extreme foreign body response and is, thus, least energetically costly. Homografts, using tissues from other individuals of the same species, are likely to invoke a greater tissue response, but may be required for smaller pearl oyster species, which have less tissue available for the autograft technique. Heterografts, using tissue from other mollusc species, invoke the greatest tissue response. Tissue from incompatible species result in a massive haemocyte infiltration response, abscess formation, tissue rupture and "rejection" of the nucleus. This response is especially costly for the pearl oyster and may render it more susceptible to additional physiological challenges (disease, environmental changes, etc.).

Intervals between seeding with artificial nuceli and mabé (half-shell pearls) production, should take into account the fitness of the pearl oyster (e.g. assessed by demonstration of somatic/shell growth) and optimum energy surfeit periods, to enhance the success of repeat surgery.

2.2.2 Hatchery production

Introduction

With decreasing wild sources of pearl oysters and increasing interest in development of stocks of consistent, superior quality, more pearl oyster producers are using hatchery-production of seed for grow out (Fisheries Western Australia, 1997). Advantages of hatchery production are reduced pressure on wild populations of pearl oysters, ability to select individuals that have optimal characteristics for pearl productivity, and reduced need for transfer of oysters from remote sites with the associated risk of introduction of new pests or diseases. Disadvantages associated with hatchery production are an increased need to handle the early, more delicate, developmental stages and the need for specialized expertise and technology for spawning and successful rearing of the larvae to metamorphosis and grow-out size. Hatchery production is also equally, if not more, susceptible to opportunistic disease problems than wild populations, but careful management and biosecurity measures can reduce this susceptibility.

Broodstock

There are no reports of health problems in pearl oysters held and spawned as broodstock, although the same problems associated with gonad manipulation for surgical implantation procedures could be expected to apply to spawning manipulations for spat production (see Section 2.2.1). Of particular significance to pearl oyster broodstock development is the need to monitor the gonadal development to determine the optimum time to induce spawning. Opening of the oyster through mechanical wedging can damage the adductor muscle and mantle margins (Mills,

Tlili and Norton, 1997), as well as the hinge. The anaesthetic, propylene phenoxetol, appears to circumvent the need to physically pry the shell open and has been reported to cause minimal mortalities, even when used on a large scale (Mills, Tlili and Norton, 1997). These authors note, however, that optimal results are only obtained for oysters which are relaxed prior to immersion in the anaesthetic solution and which have been cleaned of fouling organisms at least 24 hr prior to anaesthesia. Handling stress reduces the gape achieved using anaesthesia and fouling organisms reduce the anaesthetic concentration. Recovery is most rapid in oysters given the minimum exposure required to provide an adequate gape for examination purposes.

In other bivalve species, repeat spawnings and prolonged holding within hatcheries have frequently been associated with outbreaks of disease. Typically, the infectious agents involved are present in the bivalves in the open-water environment, but in closed-circulation facilities, can proliferate to abnormally pathogenic levels of infection (Whyte, Cawthron and McGladdery, 1994a, b). Control of such infections usually involves modifying husbandry practices to reduce physiological stress and prevent a build up of potential pathogens on tank surfaces and in pipelines (Elston, 1984). Chemotherapeuticants can be applied, but the expense of repeated applications against ubiquitous marine organisms, the risk of development of drug-resistant pathogens, and potential adverse environmental effects (see papers on Chemotherapy in Shariff, Subasinghe and Arthur, 1992), usually make alternative strategies more attractive. Examples include moving animals to disinfected tanks, reducing stocking densities, food concentrations, and temperatures, and increasing monitoring and removal of mortalities.

Although infectious agents usually build-up within the holding system, another source of potential contamination is the algal food supply. Most hatcheries are supplied by filtered seawater, in order to minimize contamination and clogging of the system by macroplankters. This means that cultured algae is necessary to provide or supplement the food required for the animals under production. Careful control of the microbial load within the algal supply and delivery system is necessary to prevent the build up of opportunistic pathogens from this source (Elston, 1984).

Another health risk associated with hatcheries is the potential for introduction of infectious agents into the hatchery system via the gut and mantle contents of the broodstock. Although these infectious agents may not affect the adult oysters, the larval offspring may be susceptible to infection. Minimizing the period of exposure of the spawning adults to their spawning products is usually effective in reducing contamination of the larvae (Elston, 1984).

Seedstock

Successful seedstock production is a challenge for any hatchery operation, since young bivalves are extremely vulnerable to energy deficits induced by competition for nutrients, toxic by-products from contaminants and microbes, as well as rapid changes in environmental conditions (temperature, salinity, pH). The concentrated somatic growth effort at this stage of development leaves little energy "buffer" for other energy demands and requires special care in provision of adequate and uncontaminated food (Krishnan and Alagarswami, 1993a, b). If anything upsets the balance between feed and growth, mortality rates occur much more rapidly than in juveniles and adult stages. Seedstock mortalities can reach 100 percent within 12-24 hr, leaving little time for remedial action. Such heavy mortalities, along with undigested food particles, produce a nutrient base for proliferation of saprobionts and secondary infectious organisms. Monitoring is, thus, of paramount importance at this stage of hatchery production (Elston, 1984). This can take the form of direct observation of the larvae themselves (velar activity), or monitoring of tank sediment/flow-through effluent particulate matter for undigested algae (indicating reduced feeding).

Hatchery techniques, aimed at minimizing the stress of handling on younger developmental stages of pearl oysters, are being developed (Rose and Baker, 1994). A monitoring study using histological cassettes to hold individual spat reduced direct contact of the fragile and convoluted edge of the developing shell and was found to have a negligible effect on larval growth (Mills, 1997). Food consumption by the larvae in cassettes was less than by free larvae, however, conversion efficiency was greater – possibly due to less handling stress. Comparisons with growth rates from parallel studies showed that *P. maxima* appear to be slower growing than *P. margaritifera* and *P. fucata* and that spat held in farm-based nurseries grow faster than those maintained in the hatchery.

Stocking density is also important for the health of the early developmental stages of bivalve molluscs. Southgate and Beer (1997) studied the effect of stocking density on black-lip pearl oysters, *P. margaritifera* grown on plastic mesh trays and in pearl nets over a 19 week growth trial. Larger spat (>10 mm) were placed in plastic mesh trays (55 x 30 x 10 cm) and, interestingly, showed greatest growth at the highest density (100 spat/tray). Spat measuring 5-10 mm were placed 7 mm mesh pearl nets and showed greatest growth at the lowest stocking densities (20 and 50 per net), which is more consistent with results from studies with *P. maxima* (Taylor *et al.*, 1997). The reason for the difference in density effect between size-groups of black-lipped pearl oysters is unclear. It is known that pearl oyster spat are gregarious and clump together (Crossland 1957; Gervis and Sims 1992; Taylor *et al.*, 1997), which can result in smothering of the internal individuals and shell deformities. The initial high growth rates observed by Southgate and Beer (1997) may reflect a growth spurt following transfer from the hatchery to open-water, and precede a slowing down when the clumps begin to fill the available space in the trays. Stocking densities which induce clumping are well documented as causing shell deformities (Taylor *et al.*, 1997) and require more handling to break the clumps apart which, in itself, may cause shell damage. This would be expected to reduce the health, quality and survival potential for the oysters as they grow-out to commercial size.

Open-water spat holding techniques with good water exchange and nutrient provision may encourage a healthy start to spat growth (Taylor *et al.*, 1997; Mills, 1997), although Rose and Baker (1994) found lower mortalities in spat reared for 5 months post-settlement in downweller facilities in a hatchery (1-2 percent mortality) compared to those in open-water plastic cages (9-12 percent). The open environment is subject to wave action and predation which, affect spat growth and health. Although suspension culture reduces losses to benthic predators (such as, crabs, starfish, gastropods), Southgate and Beer (1997) noted the presence of the fish *Paramonacanthus japonicus* ("leatherjacket") in the pearl nets and trays used in their experiment. These fish graze on the soft margins and growth processes of the shell and may also attack mantle margins. Fish, as well as other predators, have also been reported from pearl producing areas in the Red Sea, Solomon Islands and Western Australia (Crossland, 1957; Sims, 1994; Friedman and Bell, 1996). Since spat are more vulnerable to predation than older pearl oysters, regular monitoring is necessary to prevent predators from building up, or getting trapped inside, pearl nets and cages. The health risks imposed by open-water challenges need to be assessed against the capital cost and expertise required for long-term holding within a hatchery.

Where open-water culture is used for spat grow-out, suspension culture plays a significant role in enhancing successful growth of *P. maxima* spat (Taylor, Rose and Southgate, 1997b). Surface waters contain a greater biomass and diversity of planktonic and particulate nutrients than deeper or bottom waters (Taylor, Rose and Southgate, 1997b).

Selective breeding

With the production of seed, the opportunity to improve the genetics of cultured stocks by selection of favoured traits, such as shell colour, productivity and disease-resistance,

presents itself (Wada, 1987). Ideally, animals selected for breeding should have demonstrable traits for survival at the sites for intended growth. Oysters from remote sites should be bred in facilities which keep them separate from local-local crosses, to minimise the chance of contamination of local stocks - both genetically or with potential pathogens (see Section 2.2.3). This also preserves the gene pool of the natural population for back-crosses if required to reverse the development of unwanted traits or reverse in-breeding (Wada, 1993; Wada and Komaru, 1994). Dauphin and Cuif (1995) describe a change in the colour of the black pearls from *P. margaritifera* which they attribute to increased mixing of different (genetically distinct) populations of black-lip pearl oysters. Selection for white shell in the Japanese pearl oyster *P. fucata martensii* was found to have an adverse effect on growth and survival, when compared with brown-white shell hybrids (Wada and Komaru, 1994). However, the inferior growth performance of the white shelled oysters was, subsequently, found to be useful in enhancing white pearl production when used as the donor tissue for implantation in hybrid recipient oysters (Wada and Komaru, 1996).

Although inbreeding problems usually take many generations to develop when starting from a "wild" broodstock, care should be taken to use a large number of parent stocks, the crosses from which should be carefully separated and followed. This is necessary to trace both good and bad traits which may develop. Many selective breeding programs - especially for fast growth - produce unexpected "side-effects", which may or may not have an adverse effect on health. Knowing the genetic line of each generation will help with the management of any such problems, should they arise.

Triploidy – Diploidy

Triploids are usually produced to ensure sterility and enhance somatic growth. In addition, diversion of energy reserves from gametogenesis may also enhance reserves available for tissue defence. There is some evidence that triploid American oysters (*Crassostrea virginica*) are faster growers and demonstrate more resistance to infectious agents than diploid individuals (Matthiessen and Davis, 1992), however, other oyster species show negligible differences in resistance (Nell *et al.*, 1994). Reversion to diploidy and mosaic triploids (Allen *et al.*, 1996) may complicate the interpretation of disease resistance and triploidy correlations. This may also result in some individuals which can reproduce, although the resultant embryos may not be viable (He, Lin and Jiang, 1996). Triploids should, therefore, be used in areas where accidental liberation can be avoided and any escapees easily controlled.

Although there is little information available on pearl oyster triploids and disease resistance, there is evidence that growth and pearl production is enhanced in triploid *P. martensii* (Jiang *et al.*, 1993). This indicates that triploid sterility may enhance soft-tissue defence mechanisms, at least in this pearl oyster species. It should be noted, however, that triploidy itself may enhance mortality (since triploidy is not a natural state). Lin, He and Jiang (1996) found significantly higher mortalities in triploids during the straight-hinge to juvenile stage of development, compared with diploids. Interestingly, embryos and adults showed no difference in rates of mortality.

2.2.3 Introductions and transfers

Introduction

Disease risks associated with uncontrolled introductions and transfers are well-recognized (Sindermann, 1986, 1991; Brock, 1992; DeVoe, 1992; ICES, 1995; OIE, 2006, 2007), especially with the increase over the last 20-30 years of hatchery-based seed production, remote setting and use of nonindigenous species for aquaculture. Disease risk assessment for any introduction or transfer of aquatic organisms requires an accurate knowledge of the health status of both the shellfish being moved and the shellfish in the receiving waters.

Drawing upon the primary literature and other information that is often not readily available (i.e., research laboratory reports, government technical reports and personal communications with colleagues), a comprehensive and worldwide synopsis on shellfish diseases of commercially important molluscs, echinoderms and crustaceans (Bower, McGladdery and Price, 1994; Bower and McGladdery 1997) and an Asia disease diagnostic guide for important pathogens of finfish, mollusc and crustaceans (Bondad-Reantaso *et al.*, 2001) were developed. A significant gap in the knowledge contained in these documents, however, is data for south sea pearl oyster infectious agents. This makes assessment of the disease risks associated with the movement of these molluscs particularly difficult. Historic movements of pearl oysters have, for the most part, been local in nature, however, increased pressure to supply seed and adult pearl oysters as local populations dwindle, is focusing attention on more remote sources and even international transfers (Benzie, 1994; Fassler, 1994; Sims and Sarver, 1994, 1996).

Local movements

Movements of pearl oysters within a region probably present minimal health risk concerns, especially if the stocks used have been moved traditionally from the source site to grow-out site, with no disease problems. The main precaution required for this established practice is that the source stock does not change its health status by becoming mixed with stocks from other areas or become depleted, resulting in collection of sub-optimal oysters.

Movements between remote areas within a country represent a greater health risk, since one population or stock may be susceptible to infection by an organism which is benign in the other stock. It is also harder logically to monitor the health status of remote oyster populations, in which case a health check of the source stock, before transfer to the farm site, as a minimum precaution is highly recommended.

International movements

No international movements of live aquatic organisms should be undertaken without a detailed evaluation of the health status of the stock being introduced. The reasons given in Section 2.2.3 also apply to international transfers but, in addition, compatibility of the source habitat with the import habitat may also need to be evaluated. If these differ significantly (temperature, salinity, turbidity, fouling organisms, etc.) the source stock may be subject to severe physiological stress with the transfer, making them more susceptible to health problems following introduction. Although there is debate over the emphasis given such risks (Sims and Sarver, 1996), these questions can be answered by precautionary introductions of trial numbers of animals maintained in quarantine with an ambient water supply (e.g. Wang, 1994). Guidelines for quarantine assessments are given by ICES (1995) and OIE (2006). Additional concerns, such as the introduction/spread of toxic algal cysts can also be addressed by quarantine introductions (Dijkema, 1995). The standard protocol for minimising the risk of adverse effects from international aquatic animal transfers is introduction of a broodstock into quarantine for spawning. Once spawned and the spat generation have reached a size where the chance of survival is stable, the broodstock should all (100 percent) be examined for infectious agents of disease concern to the importing waters. If the broodstock are free of such agents, the spat generation can be checked. If they are also free of infectious organisms of concern, they can be released from quarantine.

Disease risk assessment

An assessment requires a detailed knowledge of what is present in both the source stock and the stocks present in the receiving site. In addition to disease, genetic and ecological impacts associated with movement of live aquatic animals should also be

assessed (ICES, 1995). The ICES (1995) Code of Practice is particularly useful for pearl oyster movements, since it aims at identification of unknown risks, unlike the OIE Code (2007) which concentrates on known disease agents. Currently, no pearl oyster diseases are listed in the OIE Code.

Ideally, a health profile (see Section 2.3) should be available for the source stock, however, such information may be rare for pearl oysters. Thus, one or more samples of the source stock should be examined for all pests and diseases before any movements to the pearl culture farm take place. Once the profile is obtained, the pearl farmer can determine whether there are any infectious agents or pests present which do not occur at the farm site. If so, the farmer is faced with the choice of finding another, more compatible, source or taking the risk of exposing the farm to potentially harmful additions. The methodologies used for health examinations are outlined in Sections 2.2 and 2.3.

2.3 DISEASE DIAGNOSTIC PROTOCOLS

2.3.1 Field collection of samples

Background information

Shellfish Health Questionnaire (Annex 2.1)

Field Data Sheet (Annex 2.2), note the following:

- hinge-lip length
- wet weight
- surface appearance (shell and soft-tissues)
- any damage to soft-tissues during opening of the shell.

Number of specimens collected (see Annex 2.3). Check the number required with the pathology laboratory and ensure each specimen is intact, i.e., no empty or mud-filled shells.

Shell surface, note the following:

- the presence of fouling organisms (barnacles, slipper limpets, sponges, polychaete worms, bivalve settlement, etc)
- shell deformities (shape, holes in the surface)
- obvious shell-fragility
- any abnormal colouration/smell
- any shell breakage or repair.

Inner shell, following removal of the soft-tissues note these observations:

- the presence of fouling organisms on the inner surface
- shell deformities (shape, holes in the surface, mud or water blisters)
- obvious shell-fragility
- abnormal colouration/smell
- pearls attached to the inner surface (cultured or wild).

Soft-tissues, note the following

- presence of abscess-like lesions, pustules or other tissue discolouration
- oedema (water blisters)
- overall transparency or wateriness
- any abnormal smell
- pearls (cultured or wild).