DR. DARIEN DANIELLE MIZUTA (Orcid ID : 0000-0002-8786-0673)

Article type : Review

Abstract

Current trends in the seafood market indicate increased demand for within-shell oysters, associated with increasing popularity of oyster bars. As this specialized, demanding market increases globally, there is strong incentive to improve quality. In this context, the manipulation of oyster shell traits through aquaculture as a means of improving oyster quality is timely and promising in terms of marketing. Several oyster shell characteristics, most especially shape, measured as length (L), width (W), and depth (D), result from a combination of three factors: genetics, environmental condition to which the oyster is exposed, and husbandry practices. Although breeding programmes have targeted several important commercial traits, selection for shell traits has not been widely performed. Additionally, accumulated knowledge of the effects of environmental conditions and farming methods on shell characteristics exists at a local level, but as such, it is not always validated with scientific data. The existing local knowledge and practices, however, are of extreme importance for the improvement and adaptation of the farming sector to market demands. Current knowledge about the genetic, environmental, and husbandry effects on shell and related aquaculture practices for manipulation of shell appearance and robustness in commercially important oysters of the Genus *Crassostrea* is compiled here. As the topic has not been well documented in the academic field, information from scientific articles was complemented by technical reports, and commercial sources. A combination of aquaculture practices is needed to produce oysters of acceptable shape, thickness, cleanliness, and colour, although suitable shell characteristics may vary with different markets.

Key words: *Crassostrea*; oyster; aquaculture; half-shell market; shell traits; shell manipulation.

Introduction

Global demand for seafood is increasing as consumers seek nutritive and healthy alternatives to a diet dominated by animal protein. Globally, molluscs are among the most important commercial groups of species in marine aquaculture, accounting for 32% (16.1 million tons). Bivalve shellfish are the main cultivated molluscs, and oysters alone account for 32% of that percentage (FAO, 2017), with the Genus *Crassostrea* representing an outstanding majority.

In recent years, oyster demand has increased mainly in live and half-shell markets, driven specially by the popularization of oyster bars (Sackton, 2013). The half-shell oyster is preferred by many consumers partly because of convenience of preparation, as it is already opened and ready-to-eat or to be further processed by barbecuing or broiling (Loose et al., 2013). Importantly, in the food industry, product appearance and presentation often matter as much as the taste itself to the consumer purchase decision. Consumers subconsciously perceive beautifully displayed food as tasting as good as it looks, and this also is true during retail display in the seafood market. In fact, the external shell appearance in live oysters is the first impression buyers have as to the quality of an oyster (BIM 1996; Brake et al., 2003). This highlights the relevance of oyster shell features as a primary determinant of acceptance in the shelled niche market. Considering the history of oyster farming that dates back to 400 BC with Greek and later Romans at the turn of the first millennium (Smith, 2015) and that oyster aquaculture has been performed in many countries with the main focus on quality of meat (Lacoste & Gaertner-Mauzoni, 2014), it was not until recently that shell appearance was recognized as a commercially important trait.

In the 1980's, a report analysed the opinions of researchers and farmers concerning goals for oyster selective breeding. In that study, shell features were not considered as a relevant aquaculture issue (Mahon, 1983). Instead, meat yield, growth, and mortality were the main selective traits to be pursued by the breeding project. Recently however, because of increased competition and emerging producer nations entering the international commerce, farmers are challenged to innovate and improve oyster traits to achieve a unique product of guaranteed quality (Brake et al., 2003). Nowadays, one of the traits adding competitive advantage to reared oysters is the enhancement of shell shape.

Shellfish are commercialised in two main formats, one being the within-shell presentation and the second shucked meat products. The first includes live and half-shell oysters, and the second involves shucking and/or processing of shellfish meats, often in the form of conserves or canned, smoked or dried (Quayle, 1988; Cheney, 2010). Usually, only shape-selected bivalves, requiring minimum post-harvest processing are sold in the shelled market for a high-end clientele; whereas, for shucked products the *in natura* oyster shell appearance is understandably irrelevant. Nevertheless, the enhancement of oyster shell shape and appearance through aquaculture practices not only improves qualitative and competitive value by making a visually-compelling appearance for general consumers and restaurants aiming at gastronomic displays, but may also improve quantitative value, commanding higher selling prices that can reach up to 60% more per oyster (as reported in Walton et al., 2012 and Leavitt et al., 2017). Accordingly, unless the target seafood market is either underdeveloped, which usually translates to lower standards and niches, or the production is destined for the unshelled market, as commonly sold in Japan (Lacoste & Gaertner-Mauzoni, 2014), the importance of the shell in commercial oysters is undeniable. Thus, methods to manipulate oyster traits can be a distinctive and critical step determining failure or success for shellfish farmers willing to compete in high-end markets and at international scales.

Despite the undeniable commercial interest, studies on shell shaping in global aquaculture are scarce. One of the reasons for this is that the shell is not as widely perceived as of mainstream importance as is the oyster meat (Ward et al., 2005). Studies on oyster soft body are numerous, but studies focusing on the shell are relatively rare. Furthermore, existing discussions about oyster shell traits consist of a few lines in articles with unrelated scope. These facts limit both the access to scientific information specific to shell morphology and the dissemination of related scientific knowledge to various stakeholders, such as commercial farmers. To provide insight on the current knowledge of oyster shells and aquaculture and fill the literature gap, this paper gathers reports with some focus on oyster shell shape, particularly considering cupped oysters of the Genus *Crassostrea*, describing farming methods currently in use to improve shell characteristics.

The purpose of this review is two-fold: 1) to review the current knowledge on factors influencing oyster shell traits; and 2) to list methods reportedly used for the manipulation of shell traits of commercial interest. Because of the aforementioned scarcity of scientific references available on the subject, it was necessary to use information derived from personal communications and technical reports. To the best of the authors' knowledge, this is the first scientific review covering methods of oyster shell manipulation. Thus, this review contributes to the dissemination of the current knowledge and culture practices related to the manipulation of oyster shell characteristics in aquaculture to assist in the establishment of higher quality standards for aquacultured oysters worldwide.

The "good" versus the "bad" oyster based on the shell

In general, the concept of attractiveness related to food may be regarded as a subjective and personal matter. For oysters however, there is consensus among shellfish farmers and the market about which characteristics define a marketable bivalve and outline the perfect shell shape (Brake et al., 2003). Oysters must be clean and hard, without sediment traces or attached epibionts (Doiron, 2008), blisters or chambers (Buestel, 2005) and within the Genus *Crassostrea*, a desirable oyster is considered to be thick, deep, and wide, with the ideal shape resembling that of a teardrop (Heath & Wilson, 1999; Handley, 2002; Doiron, 2008). On the contrary, long and skinny oysters (commonly referred to as 'bunny rabbits', 'bananas,' or 'sneakers') are considered undesirable both by market experts and farmers for being marketwise unsuitable (Kube et al., 2011).

The main characteristics of a shell (Figure 1) are: length (L), width (W), and depth (D), which allow for the classification of oyster quality based on shape, either on pre-defined values of a single dimension or combinations between two or all aforementioned measurements. Although general shape is a species characteristic, a fact that allows for an easy distinction between one and another, distinguishing intra-species quality classes of oysters based on shell appearance alone is a more difficult task.

Several quantitative descriptions of length, width, and depth have been used to categorise bivalves from the same harvest into different quality classes (Wada, 1986; Galtsoff, 1964; Imai & Sakai, 1961). Caution is necessary, however, when using absolute measurements individually, as they usually overestimate or underestimate the classification of oyster batches according to influences of different animal sizes on shape (Brake et al., 2003). Instead, simple indices such as ratios of depth/length (D/L) or width/length (W/L) can produce better results of oyster quality classification, although their use may still be limited because reference values for quality classification are still undefined. As a result, shellfish originating from different producers can result in misleading disparities in shell classifications (Kube et al., 2011).

Nevertheless, for Pacific oysters (*Crassostrea gigas*, Thunberg, 1793), desirable shell trait ratios have been described as a depth/length of 1:3 or D/L > 0.25 and a proportion of width/ length of 2:3 (0.67) or W/L > 0.63 (Brake et al., 2003; Kube et al., 2011). Shell quality classification ideally should combine both depth and width, as these two dimensions are important as determinants of good shape; however, in previous studies wherein both D/L and W/L ratios were simultaneously applied to classify oyster quality, the percentage of correct assignment reached only 30.0%, compared to much higher numbers of 85.6% and 70.6%, when D/L or W/L ratios were used alone, respectively. The substantial decrease in correct quality assignments indicated that a more reliable shell-quality index was needed. Currently, in Australia, farmers are targeting a ratio of 3L: 2W: 1D as the optimal oyster shape.

In contrast, in Canada, oyster quality is determined after harvest, during grading. For Eastern oysters (*Crassostrea virginica*, Gmelin, 1791) cultured on the bottom or harvested from natural reefs, simple measurements of the inverted width index (L/W) allow the commercial classification of oysters into 4 groups, ranging from small “cocktail” oysters to increasingly larger sizes, in sequence: “choice”, “standard”, and "commercial" (Doiron, 2008; Figure 2). In this same area, oyster yields resulting from suspended culture falls into the categories of either "cocktail" or "choice", showing that suspension-reared oysters exceed wild caught or bottom-cultured bivalves in shape quality. Automated shape quality graders that can also detect banana-shaped oysters, broken shells and other irregular shapes using computer-based image analysis also are available for the shellfish aquaculture industry (see for example: Heath & Wilson, 1999; Xiong et al., 2010) but require calibration for optimal shell shape thresholds which are still not standardized in the shellfish industry.

The characteristics of a good shell extend beyond an optimal, predefined shape, as good oysters should also have a relatively thick and robust shell to endure mechanical shocks during culture management, sorting, transportation, typical rough handling, such as shovelling and dumping (Wheaton & Hall, 2007), and mechanic processing, without sustaining undesirable shell breaks. Breaks allow the leaking of the extrapallial fluid, a major contributor to an oyster's taste, also leading to bivalve mortality; whereas thicker shells increase oyster shelf life and durability as live products (Wheaton & Hall, 2007; Doiron, 2008; Davis, 2013).

Factors influencing oyster shell characteristics and manipulation methods

Oyster shell appearance and robustness results from a combination of 3 factors: 1) genetics; 2) environmental conditions in the farming site; and 3) husbandry practices (Carriker, 1996). Genetic improvements of oyster quality have been performed through selective breeding programmes and are not a novel topic, but selection for shell traits have been only moderately explored. The latter two factors have not been explored in depth, as noted by Kube et al. (2011), who emphasised the necessity of understanding how the effects of local environment at the farming sites and culture methods influence shell shape. Although it is undeniable that environmental conditions of coastal farming sites are difficult to control (see for example Mizuta et al., 2014), and water quality characteristics are factors that are uncontrolled and may play a role in allometric growth, both farming locations and methods can influence shell aspects to a relative degree. The following sections discuss all 3 influential factors to shell traits and associate farming practices employed for shaping shells.

Genetics

Selective breeding

Shellfish have been shown to be responsive to genetic selection, which is used as a means to improve quality (Cheney, 2010). In selective breeding, selected strains are used to produce bivalve seed with desirable economic characteristics for shellfish farms. The breeding goals are based on economic analysis of oyster production and marketing (Mahon, 1983); thus, selected strains have the most important economic and commercial traits. Selection of traits should be based on the knowledge of hereditability values, for which heritability (h^2) is the portion of the phenotypic variance for a trait that is of an addictive nature, so that traits with a high hereditability index are more suitable for successful selection (Matson, 2010). Although growth, resistance to disease, and meat yield are usually considered the most important breeding program goals, the necessity of maximizing profitability suggests the opportunity to explore additional candidate traits in breeding programmes, such as shell characteristics (Wada, 1986; Cheney, 2010). In Australia, for oysters of the species *C. gigas*, which ranks as the top most important reared oyster, the economically important traits for selective breeding were growth rate, mortality, uniformity, and shell width index (W/L; Kube et al., 2011).

Shell shape is partially genetically determined (Ward et al, 2005), and studies of selection for shell morphological characteristics showed high hereditability estimates (Toro & Newkirk, 1991). Shell characteristics such as shape, colour, smoothness of the hook in the hinge (location shown in Figure 1), increase in cup concavity, and reduction in roughness were considered to be promising traits to be pursued by breeding methods (Cheney, 2010), although until recently research has mostly been done on shell growth and colour. Studies of adult-sized *C. gigas* during grow-out provided hereditability estimates for shell dimensions of varying values, such as $h^2 = 0.25 \pm 0.08 - 0.49 \pm 0.25$ for length, $h^2 = 0.23 \pm 0.08 - 0.36 \pm 0.19$ for width and $h^2 = 0.14 \pm 0.05 - 0.45 \pm 0.23$ for depth (Ward et al, 2005; Wang et al., 2012; Kong et al., 2015; Li et al., 2017), (for a detailed description on hereditability principles see Falconer, 1996). Such variance in hereditability most likely is attributable to different gene frequencies in a specific population, different strains, and the conditions of culture or management (Falconer, 1996; Li et al., 2017). All values, however, fell within the medium-to high-hereditability range if the averaged scope is considered. For a different species, the highly-valued Kumamoto oyster (*Crassostrea sikamea*, Amemiya, 1928), realised hereditability for shell length also was described as positively related to growth and relatively high at different culture stages in the same environment, with values of 0.17 ± 0.02 for larvae, 0.25 ± 0.04 for spat, and 0.33 ± 0.07 during grow-out (Zhang et al., 2016). With respect to allometric measurement, D/L in *C. gigas* is not highly heritable by the offspring (Matson, 2010); whereas, W/L has a hereditability value of approximately 0.4 (Kube et al., 2011). Genetic correlation between shell length, width, depth and wet weight traits were shown to be all positive, meaning that desired levels of a shell trait are positively associated with levels of another trait (Newkirk, 1996; Kong et al., 2015). The best genetic correlation, however, is known to be between shell length and wet weight (0.79 ± 0.25), indicating selection for weight could be performed on shell length (Kong et al., 2015).

Beyond shell shape, there have been expectations that oyster shell colour polymorphs could be of importance for the shelled market (Brake et al., 2004), but to date demand has been reported only in localised markets. Often, oyster colour has been highlighted as a commercially-important trait by the aquaculture industry in publications focused on shell variants (Feng et al., 2015). In contrast to this academic opinion, shell colour has been ranked low by farmers reporting on desirable goals for breeding selection (see Ward et al., 2005, Table 1.1). Instead, mantle edge, which seems to be positively correlated with colour of adjacent shell (Imai & Sakai, 1961; Brake et al., 2004; De et al., 2018), was reportedly preferred locally by South Korean consumers (Kang et al., 2013). In Tasmania, Nell (2001) reported a niche market for golden-shelled Pacific oysters, but again in connection to the mantle colour. Cheney (2010) described the "preference on the US East coast market of a lighter shell colour of the Eastern oysters to the colours usually expressed by the wild-Pacific oysters", which most likely stems from an association between shell and flavour and shell and type of oyster than shell colour *per se*, as locally-produced Eastern oysters are preferred in the US East market over the Pacific oyster (Lipton & Kirkley, 1994; Mackenzie, 1996;

Messer et al., 2017). Nevertheless, the possibility of considering colour as a commercial trait fostered numerous genetic studies on shell pigmentation, especially with *C. gigas.* Shell pigmentation research in Pacific oysters identified a high, narrow-sense hereditability (h^2=0.59 ± 0.19) for that trait and a dominance of a light (white colour) allele over the dark (black colour; Evans et al., 2009), but with a golden shell colour strain dominant over white, with a masking effect on black pigmentation (Ge et al., 2015). Different colour strains have different hereditability values, as shade varies from near-white (h^2 = 0.156 ± 0.08; De et al., 2018), to gold (h^2 = 0.270±0.086; Jianlong et al., 2016), near-black (h^2 = 0.52 ± 0.20 – 0.69 ± 0.16; Xu et al., 2017), and even purple, which was identified in some studies (see Song et al. 2017 for details on genetic differentiation). Genetic correlations between shell colour-related traits and growth-related traits are inconsistent, varying with colour, e.g.; golden shell traits were not correlated with growth (Wan et al., 2017), but a black shell strain seems to have a high correlation (Xu et al., 2017).

Selective breeding has effects on the cost of production of oysters. A model study was performed in Australia to analyse how much the selected traits from a selective breeding program could minimize the cost of oyster culture and increase farmer profits. Selection for shell width, identified as the ‘shell shape’ metric in that study, was found to decrease by $0.11/dozen Australian dollar (AUS) the cost of oyster production when the index was genetically improved by 10%. By means of comparison, selection for growth rate, one of the most common selective breeding goals that is directly related to the length of grow-out period, minimized the cost of production in AUS$ 0.09/dozen when grow-out period was reduced by 10%. Calculation of economic weights (expressed as a change in economic value for a unit change in the studied biological trait) for width index in that model required inputs of an average width index for a specific population (for example, a farm) and a minimum threshold of acceptable width index according to industry standards. As the economic weight for width index was highly sensitive to those, knowledge about oyster shapes resulting from specific environments and husbandry practices, and the shape quality standards in the current market are crucial data for the correct estimation of economic gains through selective breeding programmes at different locations (see Kube et al., 2011, Chapter 2 for further details).

Despite the positive gains expected on the final yield attributable to genetic selection, usually the selection of multiple strains implies complex genetic trade-offs (Kube et al., 2011). A problem would arise if a negative correlation exists between two commercially important traits, thus the need for more studies that could prevent failure of selection to yield a superior product (Newkirk, 1996). Regardless of Newkirk’s (1996) findings about positive correlations, in another study, successful selection for growth rate in Pacific oysters resulted in decreased shell width index and misshapen oysters that were unsuitable for the market. Those bivalves required either additional periods of culture to grow back into shape or were culled (Kube et al., 2011).

Shell traits reportedly able to be selected may be offset by husbandry and not revealed in the offspring. Matson (2010) discovered shell selection traits were only displayed in oysters when culture densities during grow-out were lower than the standard commercial levels, which highlights the importance of husbandry effects on shell phenotype, in addition to genetics. When discussing practical issues, the same author argued the likely higher production cost imposed by reducing stock density during grow-out, can be counterbalanced by higher selling prices and by the product classification as “premium”, with different quality standards than that of a non-selected oyster.

In addition, some bivalve characteristics such as shell depth and colour, despite having a genetic basis, are affected by local environmental condition (Imai & Sakai, 1961; Batista et al., 2008). Therefore, shellfish harvests originated from identical seed genotypes submitted to different environmental conditions may result in different phenotypes. Thus, not surprisingly, selective breeding programmes are used as a complement to husbandry methods and should not necessarily supplant physical methods currently in use in the commercial sector (Matson, 2010). Breeding programmes should arguably not be adopted as the only approach to assure satisfactory outputs of a crop (Mahon, 1983; Kube et al., 2011).

Ploidy

The genetic manipulation of oysters with regard to ploidy also may have an effect upon the resulting shape of cultured oysters. Triploid oysters differ from diploid oysters by the three sets of chromosomes, as opposed to the normal two sets, that inhibit reproduction during the warm seasons and produce a plump, glycogen-rich content oyster throughout the year (Toba, 2002) which outperforms reproductive diploids. Triploid oysters have been characterised as superior to diploids in all production metrics and also as “better looking.” In Eastern oysters, all shell metrics and shell cup ratios (D/L) have been found to be higher in triploids than in diploid counterparts (Dégremont et al., 2012; Walton et al., 2013). Although farmers believe that increases in triploid shell length result in thinner shells, a recent study reports, in addition to length increments, higher wet weight and dry shell weights in triploids (Walton et al., 2013). It is not surprising that triploid oysters have triggered interest among farmers, and triploid seed is in widespread use in markets in the US, Canada, and Europe (Nell, 2002, Buestel, 2005; Murray & Hudson, 2012).

As it is not uncommon to have both different environmental characteristics and different husbandry methods in use within a farming area, which would affect the characteristics of oysters even if that population had been genetically managed (Kube et al., 2011; Walton et al., 2013; Melo et al., 2016), solid scientific knowledge on how these two factors affect shell characteristics in oysters (included in the next sections in the present review) is crucial.

Environmental conditions

Shell traits, especially shape, differ depending on the environment to which the oyster is exposed during grow-out (Carriker, 1996). As environmental characteristics vary in time and space, oysters reared in the same locality can exhibit shell morphology variations (Seed, 1968). In commercial farms, intra-site environmental variability is very common, and oysters with differences not only in growth but also shape are harvested within a single farm resulting from conditioning in slightly different locations. Some of these factors are presented next.

Water depth and energy

Culture depth -- at surface, middle water or bottom -- and location, although usually dependent on the chosen rearing method, has an effect on the oyster shape associated with hydrological characteristics and energy of the environment at that particular depth.

Water movement is considered necessary for the shaping of oysters (Brake et al, 2003; Ward et al, 2005), oysters cultured close to the water line, where they are exposed to a high-energy environment with constant wave disturbances, have the rim of the shell frequently broken. As new shell replacements need to be built, the shells that result are relatively deeper than longer (Orton, 1936), a positive gain in terms of marketing quality.

In suspended culture, mainly performed in the middle layers with the use of longlines with net lanterns or single dropper ropes with oysters, fast growth is promoted with increased contact area with the surrounding water, compared to shellfish living in the sediment and constant submersion, compared to intertidal sites (Bishop & Peterson, 2006). The position in the water column enhances survival because of lower availability of predators and reportedly improves meat content when compared to intertidal sites where oysters would be exposed frequently to air (Johnson & Smee, 2014). Some disadvantages of the method, however, include susceptibility to higher levels of biofouling and increased shell deformities (Marshall & Dunham, 2013). Moreover, faster growing oysters cultured in suspension generally have fragile and friable shells when compared to bottom cultured oysters (Toba, 2002). Friable shells very often sustain damage during cleaning and transportation resulting in product losses and lower marketability (Robert et al., 1993; Toba, 2002).

Wild oysters living on the sea floor are known to have different shapes depending on the energy of the environment and the nature of the sediment, and the same is expected for on-bottom reared oysters. In wild reefs of the Portuguese oyster *Crassostrea angulata* (Lamarck, 1819), Orton (1936) noticed differences in the position of the mantle depending on the local sediment. In muddy seashores, the mantle would extend upwards for the shellfish to be able to expose itself easily to food particles in the water column, resulting in long and narrow shells, which also was noted by Galtsoff (1964) and Quayle (1988). In contrast, in deeper areas with less mud or in gravel, oysters showed broader shells and uniformly extended mantles, and according to Quayle (1988) tend to be rounder and deeper. To improve the shape of bottom-cultured oysters, individuals can be set sparsely on hard, pebbly ground where they are likely to roll and move, particularly during stormy weather, so that they can become round and deeply-cupped with a degree of fluting on the right valve (Galtsoff, 1964; Quayle, 1988; Toba, 2002).

The combination of high water movement in locations with fine bottom sediment increases turbidity and induces the production of thicker shells in oysters growing in these locations. Fine particles can be allocated in chambers created between the many layers composing shell, a natural process in waters with periodically high suspension of fine sediments (Higuera-Ruiz & Elorza, 2011). This, however, is unlikely useful for the farming industry, as these conditions compel oysters to expend energy separating inorganic particles from organic particles at the expense of feeding (Quayle, 1988).

Culture location relative to the distance to the shore, as in tidal zones, also influences oyster shape. Oyster shell thickness and strength are expected to be dependent upon the harshness, namely energy, of the culture environment (Mehrubeoglu et al., 2013). Oysters farmed in shallow, coastal, highly-energetic environments, being under the action of waves in the intertidal zone, are different from oysters farmed in the subtidal zone (Orton, 1936) both in shell texture, shape and thickness; characteristics which were portrayed in field experiments conducted with non-destructive hyperspectral and thermal imaging and digital photography (Mehrubeoglu et al., 2013). There is a tendency for increased shell smoothness and thickness nearshore. Based upon this, different culture locations with different energy levels can be explored to benefit the final shellfish product. Indeed it is a common practice in Australia to take advantage of tide changes to promote constantly movement of oysters, as will be discussed further in the item “Husbandry” in this review.

Biofouling

Fouling is a major problem as it affects both apparent oyster shape, can leave permanent, unsightly scars on the shell, and can vary widely, in quantity and species, with location (Wheaton & Hall, 2007; Doiron, 2008). In the marine environment, aquaculture structures as well as the cultured bivalves themselves provide substrate for settlement of larvae of a number of marine organisms. Some of the most common biofoulers on oysters are sea squirts or Ascidians, barnacles, hydroids, macroalgae and other bivalves (Quayle, 1988; Adams et al., 2011). In oyster farming, incrusting organisms affect operational economic costs because of the necessary and time-consuming cleanings and changes of culture apparatus to alleviate increasing weight loads. Attaching organisms, collectively termed epibiontes, also affect hosts as they may reduce bivalve growth and decrease shell visual aspects (blisters, erosion), on both exterior and interior surfaces (Marshall & Dunham, 2013). To the farmer, this means economic losses because heavily fouled oysters are often shell deformed. In the case of oysters containing the mud-worm shell borer, *Polydora* spp., the worm-induced internal shell blisters are visually unappealing, making oysters unsuitable for the shelled market (Figure 3; Taylor et al., 1997; Handley, 2002). On the other hand, boring sponges make bivalve shells porous as they use the host shell as a home by boring holes of approximate 1 mm on the exterior shell surface (Doiron, 2008). Additionally, biofouling can not only reduce water flow inside the farming apparatus but also compete for food with the oysters because many fouling organisms are also suspension feeders (Gosling, 2004) and as such, they can hinder oyster development and lead to undersized shells and slender meat.

Although biofouling control can be costly, the negative effects it can have on oyster marketability if not controlled can be even more expensive, to the extent that mitigative management should be a priority (see review in Lacoste & Gaertner-Mauzoni, 2014). In a survey about bioufouling perception and implications to the shellfish market performed in different locations of the United States, up to 43.4% of farmers stated that biofouling affects the marketability of their products (Adams et al., 2011). Two of the main reported commercial issues were unsightly shell appearance (57.3%) followed by resulting increased product rejection by the buyers (21.4%; Adams et al., 2011).

In view of the aforementioned marketability issues, and because biofouling is maximized in subtidal suspended cultures, the most popular culture methods, the development and dissemination of different techniques for containment of fouling species are necessary (Marshall & Dunham, 2013). Some of the most widely employed methods for biofouling removal or 'punishment', as they are popularly called, include manual cleaning and scraping, pressure washing, fresh water baths, and air exposure (Quayle, 1988; Nel et al., 1996; Handley & Bergquist, 1997; Cheney, 2010). The first three methods are mechanical and labour intensive, but the last two immersion methods take advantage of the fact that oysters can close their valves and isolate tissues from the external environment; whereas, most epibiontes do not have this ability and are killed by the immersion in freshwater or dehydration (Quayle, 1988).

Similarly to freshwater baths, oysters exposed to natural freshwater discharges are provided with food and protection from predators and biofouling arising from freshwater-induced salinity variation in this environment (Oczkowski, 2011; Pollack et al., 2011). Notably, oysters grown in suspended cultures near freshwater discharges have higher meat content (Mizuta et al., 2014). Culture areas that are located near small rivers, therefore, can take advantage of both natural biofouling control and food availability.

Nevertheless, control methods are usually combined to target differentially occurring biofouling in a local area. For instance, freshwater baths kill most epibiontes but marine worms that drill and inhabit holes in oyster shells require stronger treatments. In biofouling control experiments wherein oysters were submersed in salt brine solution (300 parts per thousand Windson Fine Salt) for as little period as 15 s, the efficiency in killing worm infestation was considerable (MacNair, 2001). Chemical treatments for biofouling control were used in the past even directly in the environment, such as quicklime powder spread on bottom culture beds to control star-fish (Quayle, 1988), but understandably, for environmental reasons, they are not popular nowadays.

More recently, different types of culture media have been tested with the objective of biofouling control in shellfish, such as clay aggregate and lava rock. These media are added to the grow-out apparatus together with the shellfish to act as abrasives that brush encrusting organisms off the bivalves when there is movement of the culture apparatus, such as with action of waves. Results showed that media slightly decreased fouling organism levels, depending on the volume of media added and targeted biofouling species, but they were not effective against tubeworm or sponges (Dunham & Marshall, 2012; Marshall & Dunham, 2013). Although worms are harmless to consumers, they not only decrease shell aesthetics but they can also be transferred to the meat in the process of shucking, prompting consumers to consider these oysters unappetizing (Figure 3).

Culture equipment has also evolved to address biofouling issues with innovative designs that allow exposing the oysters to air *in situ*. Equipment like the floating oyster cage Gro®, currently commercialized by an American company, consists of plastic floats attached to a metal framed cage that secure submersion just below the water surface and, when turned upside-down, allows flotation of oysters above surface for air-exposure to target biofouling control (Figure 4).

Predator influence

Several recent studies show oysters have the intrinsic ability to change shell characteristics in response to predators in the environment. In experiments with oysters grown in two different sites, namely with predators such as mud or blue crabs and no-predator control sites, juvenile oysters allocated more energy toward shell growth when a predator was present, at the expense of producing soft tissue (Peterson, 1986; Johnson & Smee, 2014). This mechanism was identified as a protective mechanism against possible predation risk, as thicker shells were harder to crush, thus more resistant against predation (Robinson et al., 2014). Yet, shell thickening was observed only in juvenile oysters (10-20 mm length), not adults (~55 mm), which reportedly do not modify shell in the presence of predators (Newell et al., 2007; Lunt & Smee, 2014).

Aquaculture is conducted with bags and netting of different mesh sizes adapted according to sizes of spat, juveniles, and adults, usually making cultured shellfish inaccessible to such predators, thus the relevance of the aforementioned natural adaptation to the culture industry is relatively low, but nevertheless informative.

Pollution

It is widespread knowledge that chemical pollution affects the physiology of animals, especially filter feeding bivalves which have been used as ecological bioindicators. Bivalves can be good "sentinels" of environmental quality because they are sessile and sensitive to chemical and physical conditions (Boening, 1999). Pollution, however, also can affect the morphology of bivalve shells, the most known example being tributyltin (often referred to as TBT). TBT is a toxic compound used as a chemical biofouling suppressant on boats; it is known to disrupt reproduction in molluscs. First reported for Pacific oyster populations sampled close to marinas in the 1970's and 1980's in France, and later confirmed by laboratory experiments in which oysters exposed to TBT were compared to oysters grown in good water quality, TBT was shown to cause several anomalies in oyster shells. Abnormal shell thickening with formation of stacked, layered chambers within the shell (such as the visual aspect of a cake made of intercalated layers of cake pastry and frosting; for details see Figure 2 in Higuera-Ruiz & Elorza, 2011), external shell surface deformities, formation of a colloid resembling jelly, and retraction of shell margin were all attributed to exposure to TBT (Alzieu et al., 1982; Quayle, 1988; Pinder et al., 1999; Bayen et al., 2007; Higuera-Ruiz & Elorza, 2011). The TBT effects in oysters seem to vary between species, as Pacific oysters were reportedly more sensitive to TBT exposure than Eastern oysters in laboratory tests. Research efforts and public attention were more directed towards effects on reproduction and population than to shell deformities (Pinder et al., 1999). For seafood safety reasons, polluted areas, including marinas, are already avoided for shellfish farming, especially in suspended culture areas that tend to be more removed from domestic or industrial pollution (Quayle, 1988), but the information described here indicates possible, visible effects of pollution upon oyster crops.

Husbandry practices

Marine farmers are interested in quickly supplying the demanding market. As a consequence, often the most commonly used farming methods favour oyster fast growth to a commercial size (usually 7-10 cm), but this decision negatively influences oyster shell shape and shellfish retail value in the shelled market. Fast growing oysters can also grow long and skinny, a shape that is rarely acceptable for sale. Culture practices to avoid shell misshaping are usually only performed in countries where oyster culture is well developed. For this reason, the dissemination of knowledge on oyster characteristics and relation to each culture practice are essential to give the producer the choice of making informed decisions on the most suitable method according to the expected final product.

Use of single seed

The most common sources of seeds for oyster aquaculture are natural spat collection and the use of single seed from hatcheries. In the first, oyster spat originate from natural spawnings and the ready-to-set larvae are collected after spawning season by placement of a collector in the water, such as a brush, crates of tiles, ropes, bamboo sticks, plastic equipment, or shells (Galtsoff et al., 1930). After collection, and having reached a reasonable size, the spat can be either removed from collectors and individually sorted by sizes to be seeded, or grown-out on the cultch as-is, which results in oyster clumps that must be broken apart following harvest (Toba, 2002). This last technique is customarily performed with the spat-on-shell, in Japan, traditionally using scallop shells strings, and resulting in market-sized oysters that sit on top of each other (Kato-Yoshinaga et al., 2014; Figure 5a). In this method, control of shell shape is impossible, and it produces unattractive oysters of inconsistent shape and size, unacceptable for the discriminating oyster diner. As a result, oysters grown by this method are most suitable for the shucked meat market, which the Japanese public is satisfied with, illustrated by the fact that 99% of the national oyster production targets this selling route (Korringa, 1976). On the other hand, single seed oysters are used in large-scale aquaculture that focuses on relatively easier culture management, increased survival rates, and quality of shell shape for the half-shell market, preferred in western seafood trades and commanding higher prices (Toba, 2002). In an experiment in Nagasaki, Japan, oysters grown by the single seed method resulted in round-shaped and “good-looking” oysters for the raw market with higher levels of amino acid associated with sweetness and umami taste than oysters cultured in the traditional spat-on-shell clustered method (Kato-Yoshinaga et al., 2014).

One of the newest approaches in single seed oyster culture to avoid shell deformities is under use in New Zealand and uses hatchery grown single seed of 30 mm glued to long-line droppers by special tags attached in the umbo in a way that oysters hang free and spaced from each other (Figure 5b; Brown, 2017). Because there is no handling involved with oysters in these systems, their genetic expression would express better than with other culture techniques (Achim Janke, TOPS Oysters Consulting Ltd, 2017; *personal communication*). The tag glued to the shell in this case does not reduce shell appearance because it is supposed to attest the origin of the product as a brand and allow for traceability.

As natural spat availability can be irregular with environmental conditions, single seed supply should preferably rely on the production of hatcheries that can provide ready-to-farm seed in a variety of sizes (Galtsoff, 1930). The use of hatchery-produced seed has both advantages and disadvantages, such as constant seed availability and relatively higher cost that fluctuates with demand. With lower cost of natural spat even in nations where aquaculture is a part of the mainstream fisheries (e.g.: France, United States, Japan), the industry requirements, as far as hatchery-produced availability of single seeds is concerned, may not be met. Notwithstanding, single seed hatchery facilities are not beyond the infrastructural capability of many countries, including developing nations. Brazil, for example, has already established hatchery resources for constant seed supply service of Pacific oysters for farmers in the main productive area in south of the country, which shows that hatchery production is more a matter of perceived importance and allocation of funds. For instance in Japan, after the Great East Earthquake and followed tsunami disaster that washed away many shellfish farms in one of the nation's most important fishery area, farmers wanted to quickly re-establish mariculture while facing several environmental issues that comprised not only food security scrutiny but also lack of seed to re-start farming (Okuda & Ohashi, 2012). Nowadays, still under a recovering process, Japanese farmers seek to embrace the new available technology that does not rely only in natural spat. The importance of single-seed availability and the resulting oysters of high quality level have been discussed in recent aquaculture meetings. Recently, farmer forums and technical cooperation exchange between international farmers were held by the World Oyster Society to discuss the single-seed importance for uniformly shaped outputs that could suit upscale new restaurants and bars (Suizan-Keizai, 2014).

Stocking density

Stocking density refers to the number of individuals placed in a culture apparatus at the same time. Overstocking is widespread believed to cause crop mortality, lower growth and, in relation to shell, irregular shape, clusters with possible fusion of one or more oyster shells, in addition to oysters with inhibited growth and low condition index -- a ratio between dry soft tissue weight and dry shell weight (Galtsoff, 1964; Quayle, 1988; Marshall & Dunham, 2013). Physical compression attributed to fast growth and high density of bivalves was argued to elongate forms; whereas, low physical compression culminated in triangular shaped shells (Seed, 1968). Therefore, allowing enough free space when stocking culture apparatus is important. Most studies focus on relationships between stocking densities and shellfish overall growth and mortality (e.g.: Bishop & Hooper, 2005 for *C. virginica* and *C. ariakensis* (Fujita, 1913); Roncarati et al., 2017 for *C. gigas*), but space favours quality of shell as it allows externally triggered movement of individuals, which enables the trimming of the shell edge when reared shellfish knock against each other, promoting the development of a cupped shape. For example, in an experiment comparing shape of juvenile Pacific oysters cultured in suspension in trays stocked at different typical commercial densities of 226, 453 and 679 individuals.m^{-2}, it was found that both W/L and D/L shell indices decreased at higher stocking densities, but density did not have a significant effect on occurrence of clustering, namely percentage of individual oysters “cemented” to other oysters during grow-out (Marshall & Dunham, 2013). Davis (2013) found that stocking density (following commercial standards of 75, 90, 105 oysters per basket) was negatively related to oyster cup and broader width and meat content, but had no effects on survival, biofouling control or shell robustness. Surprisingly, shell length was higher the higher the stocking density, but at a cost of shell shape because oysters were, as aforementioned, significantly less cupped and fanned.

Stocking density effects were also investigated for other less popular species. In hatchery cultures of the Cortez oyster spat (*Crassostrea corteziensis*, Hertlein, 1951) growth in shell length, total volume and wet weight significantly decreased with progressively higher stocking density (low = 5714 , medium = 11428, and high = 17142 specimens) in upwelling cylinders used for culture and kept at the same conditions (Mazón-Suástegui et al., 2008). Interestingly, higher densities allowed for the best shell length performances in mangrove oyster seeds (*Crassostrea rizophorae,* Guilding, 1828) cultured in suspended lanterns (400 mm in diameter), although the authors did not hypothesize on possible explanations for their results. The oyster seeds stocked at high density (2,000 seeds/ lantern level) achieved higher shell length than the low density treatment after 5 months and cleaning management of 14 days as opposed to 7 days, with growth of 9.9 mm/ month (Maccacchero et al., 2007).

Nevertheless, the negative effects of overstocking seems to be such a mainstream perception among farmers that it may have triggered underexploited leases in Canada. Based upon surveys, Comeau (2013) estimated stocking densities at oyster farms (0.5 ± 0.1 kg oyster.m^{-2} for floating cages and 1.0 ± 0.1 kg oyster.m^{-2} for direct bottom culture areas, compared to natural reefs of 2.2 ± 1.1 kg oyster.m^{-2}) and found densities are below the capacity indicated in related farming guidelines, a fact that was exacerbated by the size of leased farm areas (larger leases tended to be significantly less densely stocked than smaller leases and vice-versa).

Tumbling

Tumbling consists of rolling oysters over and over, to and fro, or end over end. Oysters are routinely tumbled as part of the farming process to increase shell strength by making it thicker and to prune shell extremities to make it more cupped, increasing shell depth and width in relation to length. Periodic tumbling of oysters leads to breaks in the shells extremities and increases glycogen storage to repair the broken extremities, thus also improving oyster flavour (Cheney, 2010). The revolving action reportedly thickens shell (Robert et al., 1993; Toba, 2002), however, oyster growth, measured as shell length, is slowed following successive breakage of shell.

There are different ways in which an oyster can be tumbled. Machine tumbling of oysters consists in running oysters at different stages, but especially juveniles, through a rotating meshed cylinder to break the oyster shell end and promote a deeper cupped shell. Although efficient, this method is time consuming because it requires the harvesting of the oysters for processing and followed re-stocking into the sea.

Tide tumbling, on the other hand, differs from the previous method because there is no machine involved in this case. Tumbling is performed *in situ* with the use of differences in tidal levels. The tumbling system consists of floats attached to one end of farming bags while the other end is attached to suspended long-lines in intertidal areas. When water levels vary with the tide, one side of the bags will move up and down with the floats, in a way that oysters are submitted to sporadic shaking (Figure 5c).

Another method of tumbling is termed flip-floating. In this method, floating buoys are attached to growing devices, usually baskets, which are aligned on both sides of surface long-line systems to allow the flipping of one basket on top of the other to expose the flipped top basket to the air. As a result, oysters are trimmed, growth can also be controlled by frequent periods of food deprivation, and the method additionally allows for biofouling control.

A recent innovation was developed in Australia with long lengthened meshed plastic baskets (such as Seapa's, BST's) that are hung from cables and enjoy free lateral movement with water movement (Figure 5d), being that currents, waves, tides. The technology can be used both in intertidal and subtidal areas. In recent studies with Eastern oysters, tumbled oysters grown in intertidal set ups with this system grew at a quarter speed of the oyster in common intertidal rack systems (0.03 mm length /day and 0.12 mm length /day, respectively), but oyster quality improved. The baskets were shown to produce deeper cupped *C. virginica* than the conventional floating bags and rack cultures based on W/L and D/L ratios, with higher soft tissue, and heavier shells when compared to static rack growing systems (41.3 g and 35.0 g, respectively, for 7.5 cm length oysters; Leavitt and Griffin, 2015; Leavitt et al., 2017). The movement provided by the baskets also decreased incidence of blister worms from 100 % (in conventional static culture) to 30 %.

Conditioning: site alternation during grow-out

It has been known that shellfish exhibit different phenotypes when transplanted to different environments (Seed, 1986). In the farming industry oysters have been conditioned for fattening, greening, such as the famous coloured-meat oysters from Marennes-Oléron Bay in France, and shape (Soletchnick et al., 2001; Thomas, 2016).Conditioning oysters for shell shape as a final stage of the culture, usually a time between reaching a suitable shell size and the harvest for sale, is routinely performed in the Pacific coast of U.S (Thomas, 2016). Oysters are grown in two distinct places as subsequent aquaculture phases: first the grow-out is performed in subtidal zones in suspended culture, then oysters are transferred to the intertidal area for bottom culture, where food is also usually readily available and wave energy is high, enabling “hardening”, cupping and fattening. In this way, the oysters supposedly develop the aforementioned characteristic quality traits of each locality.

Metal rings for shape innovation

Originality has always had a place in competitive markets. A new method developed in farms in Nagasaki Prefecture, Japan, explores the *in natura* oyster market by producing heart-shaped oysters for commemorative days such as Valentine’s Day. The method consists of shaping juvenile oysters using metal rings that apply pressure to a specific point of the shell to split the shell extremity to resemble a heart shape. In this case, oysters are directed to distinguished sales, mostly restaurants (Mizuta & Vlachopoulou, 2017). The distinguished shape is also being used as a market strategy in Leucate region, south of France (Agence France-Presse, 2017). Although possibly not a management that can be adopted worldwide, the development of shell shaping technology to cover specific demands and local market opportunities is valid and should be pursued.

Discussion

Aquaculture production has been increasing exponentially in the last decades, and as demand and competition increase the quality levels are raised. Aggregation of value is considered a good commercial strategy to improve a company’s level of competitiveness (Blackstad, 1995). With the ascension of the within-shell oyster market, shell appearance is an additional attribute defining oyster quality and is currently commanding higher prices. Poor shell quality may result in unsuccessful attempts to enter demanding or international seafood markets by farmers.

To satisfy new quality standards, farmers can choose among genetically selected seeds, appropriate sites with respect to environmental conditions, and grow-out methods, summarized in Table 1. Even so, these choices are to some extent linked to each other, as the characteristics of a farming site accommodate only specific types of husbandry methods and vice-versa, and when mariculture sites are more than often defined by regulators instead of freely chosen by farmers (Walton et al., 2013). Furthermore, as genetic improvement functions to complement aquaculture management activities, it is uncommon to be employed as the only method to achieve successful oyster shell quality improvement. Considering also the fact that trimming oyster shells seems to be essential to produce a satisfactory oyster, presumably, “shell shaping”, in some cases, translates into additional management efforts as well as longer grow-out periods before harvest, even in cases when selected breeding has been performed. The added value to the final product, as well as the positive competitiveness in a niche business, suggests advantageous trade-offs. Notably, recently-available technology such as the floating cages that allow *in situ* air exposure so there is no need to bring cages to shore for desiccation, or flip-floating bags, which tumble and allow air exposure independently of water levels, contribute to minimise the required labour and time devoted to shaping oysters. Because aquaculture technology is expected to develop and constantly improve as the activity expands, shell-shaping tends to become simpler and more feasible.

In addition to the added market value derived from shell shaping, the practice is rewarding because it brings additional benefits to the final product, such as improved perception of the meat, protection against predators, ease during grading and even longer shelf-life. For instance, oyster grown into the ‘right’ shape has cupped shells that allow meat to set in perfectly, thus conveying a plump visual aspect of the meat when shucked (Garry Thompson, Seapa, 2014; *personal communication*). Besides, shell shape can influence the ability of bivalves to protect against predators that bore into the shell to access internal oyster tissue, as curvature of the shell (described as depth) was found to be positively related to the compressive strength necessary to produce a crack on the shell (Lombardi et al., 2013). The revolving action of in culture apparatus that allows oyster shell tumbling were also found to promote fast recovery of oysters, in terms of meat content, after the spawning period, which is a critical time with major mortality events (Robert et al., 1993). Wheaton & Hall (2007) and Kube et al. (2011) also noted that the high variability in the shape of oysters, which is exacerbated by fouling, limited the development and widespread application of automated shucking machines that would produce oysters on the half shell. Although progress has been made in performance of automated grading machines (see Xiong et al., 2010), single bred, clean, uniformly-shaped bivalves would allow better machine-shucking, saving time and on work that is tedious, demanding, and dangerous to “shuckers” (Wheaton & Hall, 2007). In relation to oyster marketing the shelf-life is very important and increased by oyster shelf thickness as it can stand higher impacts during handling and transportation. The addition of final stage of farming, essentially the conditioning or hardening of oysters, can provide such aforementioned results and additionally has the potential of strengthening the adductor muscle preventing shell gape, which also increases shelf-life (Toba, 2002).

The possibility of combining a variety of methods would expectedly produce better results, but methods should be chosen in accordance to the main shell targets and experimental trials should ideally be conducted for each selected farming area to assure expected objectives are able to be met. Since the adoption of one method over the other may result in different costs and profits, future economic studies comparing expected methods for shell manipulation can also assist in aquaculture management decisions. Nevertheless, farmers can also choose to satisfy both shelled and shucked markets, using for the latter the underperforming, suboptimal-shaped oysters or even a parcel of the crop that was purposely not at any stage managed for improved shape.

Despite of the aforementioned, first and foremost, the adoption of standard thresholds for the evaluation of oyster shape and assessments of consumers’ preferences would facilitate the shellfish grower’s compliance with requirements of the shelled market. The lack of ideal shape thresholds was already exposed by Brake et al. (2003) and more than ten years later the problem still persists, making it difficult for the farmers and consumers to agree in a common shape classification at regional and international levels.

Although it is unlikely that some of the topics in this review, such as predator influence, could be used as a means of manipulating oyster shell characteristics to satisfy market requirements, it is important to acknowledge and publicise the wide-range of possible mechanisms that could hinder expected shell results. Additionally, the compiled information may lead to new observations and formulation of hypotheses by both industry (farmers) and science (researchers) that can be scientifically investigated and proved or disproved in the future, helping develop knowledge and practices in the field.

Until the present, much of the reported knowledge on oyster shell manipulation through aquaculture and related secondary effects still lacked solid scientific validation, as evidenced by the aforementioned shell colour importance or increases in glycogen and wet weight attributed to shell trimming. Therefore, the knowledge reported in this review should be interpreted with caution as future studies are needed to test reported assumptions on shell manipulation and possible positive and negative side effects. Nevertheless, shaping oysters is legitimately feasible, and most techniques are readily available for farmers. These contemporary aquaculture practices represent a worth-while opportunity for small and large farming enterprises to explore a growing niche market and build trust with the exigent seafood aficionado.

Acknowledgements

The authors would like to thank Mr. Garry Thompson from Australian company Seapa aquaculture devices for sharing his knowledge. Special thanks to the Bouctouche Bay Industries with the OysterGro technology, for the Department of Agriculture, Aquaculture and Fisheries of New Brunswick, for Mr. Achim Janke from TOPS Oysters Consulting Ltd and Taylor Shellfish Farms, and for Mr. Matt Will from Seapa US for authorizing the use and reproduction of their photos/schematic representations in this article. This research was supported by the American National Academy of Sciences, Engineering and Medicine through the Research Associateship Program.

References

Adams, C.M., S.E. Shumway, R.B. Whitlatch, T. Getchis (2011) Biofouling in marine molluscan shellfish aquaculture: a survey assessing the business and economic implications of mitigation. *Journal of the World Aquaculture Society* **42** (2), 242- 252.

Agence France-Presse (2017). On this Valentine's, surprise your beloved with a tasty offering: a heart-shaped oyster. Available at: https://www.ndtv.com/offbeat/on-this-valentines-surprise-your-beloved-with-a-tasty-offering-a-heart-shaped-oyster-1658461.

Alzieu, C., M. Heral, Y. Thibaud, M.J. Dardignac, M. Feuillet (1982) Influence des peintures antisalissures à base d'organostanniques sur la calcification de la coquille de l'huître *Crassostrea gigas*, *Revue des Travaux de l'Institut des Pêches Maritimes* **45** (2), 100–116. (*In French*) Bastista, F. M., R.Ben-Hamadou, V.G. Fonseca, N. Taris, F. Ruano, M.A. Reis-Henriques, P. Boundry (2008) Comparative study of shell shape and muscle scar pigmentation in the closely related cupped oyster *Crassostrea angulata*, *C. gigas* and their reciprocal hybrids. *Aquatic Living Resources* **21**, 31-38.

Bayen, S., H. Keehee, J. Obbard (2007) Exposure and response of aquacultured oysters, *C. gigas*, to marine contaminants. *Environmental Research* **103** (3), 375-382.

BIM (1996) BIM – Industry code practice for quality Irish oysters. *An Bord Iascaigh Mhara, Dun Laoghaire, Co. Dublin. Ireland.*

Bishop, M.J., P.J. Hooper (2005) Flow, stocking density and treatment against *Polydora* spp.: Influences on nursery growth and mortality of the oysters *Crassostrea virginica* and *C. ariakensis*. *Aquaculture* **246**, 251-261.

Bishop, M.J., C.H. Peterson (2006) Direct effects of physical stress can be counteracted by indirect benefits: oyster growth on a tidal elevation gradient. *Oecologia* **147**:426–43.

Blackstad, F. (1995) Outlook for aquaculture industry profiting from improved quality control in aquaculture. Paper presented at the EUROFISH Report Trade Conference "Increasing Demand vs. Diminishing Supply". Brussels, Belgium, 15-16 November 1995.

Boening, D.W. (1999) An evaluation of bivalves as biomonitors of heavy metals pollution in marine waters. Environmental Monitoring and Assessment **55** (3), 459-470.

Brake, J., F. Evans, C. Langdon (2003) Is the beauty in the eye of the beholder? Development of a simple method to describe shell shape for the Pacific oyster industry. *Journal of Shellfish Research* **22** (3), 767-771.

Brake, J., Evans, F., Langdon, C. (2004) Evidence for genetic control of pigmentation of shell and mantle edge in selected families of Pacific oysters, *Crassostrea gigas*. *Aquaculture* **229**, 89–98.

Brown, J. (2017) TOPS crops: novel development from New Zealand. *Fish Farmer* **40** (2), 22-23.

Buestel, D. (2005) History, Status and Future of Oyster Culture in France. Proceedings of the 1st International Oyster Symposium. Oyster Research Institute News 20. Available at: https://pdfs.semanticscholar.org/d2d6/178356cf60e4c938535ec8320a3c9db61b7b.pdf

Carriker, M. R. (1996) The shell and ligament. In: *The eastern oyster, Crassostrea virginica*. In: Kenedy, V.S., R.E.I. Newell, A.F. Eble (eds.), pp. 75-168. College Park, MD; Maryland Sea Grant College Publication.

Cheney, D.P. (2010) Bivalve shellfish quality in the USA: from hatchery to the consumer. *Journal of the World Aquaculture Society* **41**, 192-206.

Comeau, L.A. (2013) Suspended versus bottom oyster culture in eastern Canada: Comparing stocking densities and clearance rates. *Aquaculture* **410-411**, 57-65.

Davis, J.E. (2013) Effects of basket arrangement and stocking density when using the adjustable long-line system for oyster grow-out. Master dissertation for the Graduate Faculty of Auburn University. Auburn, Alabama, 81p.

De, X., Q. Li, L. Kong, H. Yu (2018) Heritability estimate for mantle edge pigmentation and correlation with shell pigmentation in the white-shell strain of Pacific oyster, *Crassostrea gigas. Aquaculture* **482**, 73-77.

Dégremont, L., Garcia, C., Frank-Lawale, A., Allen Jr., S.K. (2012) Triploid oysters in Chesapeake Bay: comparison of diploid and triploid *Crassostrea virginica*. *Journal of Shellfish Research* **31**, 21–31.

Doiron, S. (2008) Reference Manual for Oyster Aquaculturists. New Brunswick Department of Agriculture and Aquaculture.

Dunham, A.,R.D. Marshall (2012) Using stocking density modifications and novel growth medium to control shell deformities and biofouling in suspended culture of bivalves. *Aquaculture* **324-325**, 234-241.

Evans, S., M.D. Camara, C.J. Langdon. (2009) Heritability of shell pigmentation in the Pacific oyster, *Crassostrea gigas*. *Aquaculture* **286**, 211-216.

FAO (2017) FAO Global Fishery and Aquaculture Productions Statistics. FishStatJ Software.

Falconer, D.S. (1996) Introduction to quantitative genetics. 4th edition, Longman Group Ltd, London. 464 pp.

Feng D, Li Q, Yu H, Zhao X, Kong L (2015) Comparative Transcriptome Analysis of the Pacific Oyster *Crassostrea gigas* Characterized by Shell Colors: Identification of Genetic Bases Potentially Involved in Pigmentation. *PLoS ONE* **10** (12), e0145257.

Galtsoff, P.S., A.F. Prytherch, A.C. McMillin (1930) An experimental study in production and collection of seed oyster. *Bulletin of the Bureau of Fisheries xvli* **46**, 197-263.

Galtsoff, P.S. (1964) Ch2. Morphology and structure of shell, the American oyster, *Crassostrea virginica* (Gmelin). *Fishery Bulletin*, U.S. Fisheries Wildlife Service, 16-31.

Ge, J., Q. Li, H Yu, L. Kong (2015) Mendelian inheritance of golden shell color in the Pacific oyster *Crassostrea gigas*. *Aquaculture* **441**, 21-24.

Gosling, E. (2004) Bivalve molluscs: biology, ecology and culture. Fishing New Books. Blackwell Science, Oxford.

Handley, S.J., P.R. Bergquist (1997) Spionid polychaete infestations of intertidal Pacific oysters *Crassostrea gigas* (Thunberg), Mahurangui Harbour, northern New Zealand. *Aquaculture* **153**, 191-205.

Handley, S.J. (2002) Optimizing intertidal Pacific oyster (Thunberg) culture, Houhora Harbour, northern New Zealand. *Aquaculture Research* **33**, 1019-1030.

Heath, P.L., Wilson, J.H. (1999) Assessment of Pacific oyster, *Crassostrea gigas* (Thunberg), size and quality using a computer shape analysis technique. *Aquaculture Research* **30**, 299-303.

Higuera-Ruiz, R., J. Elorza (2011) Shell thickening and chambering in the oyster *Crassostrea gigas*: natural and anthropogenic influence of tributyltin contamination. *Environmental Technology* **32** (6), 583-591.

Imai, T., S. Sakai (1961) Study of breeding of Japanese oyster *Crassostrea gigas*. *Tohoku Journal of Agriculture Research* **12**, 125-171.

Jianlong, G., L. Qi, Y. Hong, L. Kong (2016) Selection response in mass selection of golden shell Pacific oyster (*Crassostrea gigas*). *Journal of Fisheries of China* **40** (4), 612-617. (*In Chinese with abstract in English*).

Johnson, K.D., D.L. Smee (2014) Predators influence the tidal distribution of oysters (*Crassostrea virginica*). *Marine Biology* **161**, 1557–1564.

Kang, J.H., H.S. Kang, J.M. Lee, C.M. An, S.Y. Kim, Y.M. Lee., J.J. Kim (2013) Characterizations of Shell and Mantle Edge Pigmentation of a Pacific Oyster, *Crassostrea gigas,* in Korean Peninsula. *Asian-Australasian Journal of Animal Sciences* (AJAS) **26** (12), 1659-1664.

Kato-Yoshinaga, Y., C. Kitaoka, A. Shinagawa (2014) Comparison of free amino acid components in the Pacific oyster reared using two different culture methods in Nagasaki prefecture. *Japanese Journal of Food Chemistry and Safety* **21** (2), 121-126. Kong, N., Q. Li, H. Yu, L.F. Kong (2015) Heritability estimates for growth-related traits in the Pacific oyster (*Crassostrea gigas*) using a molecular pedigree. *Aquaculture Research* ***46***, 499-508.

Korringa, P. (1976) Farming the cupped oysters of the genus *Crassostrea*. Developments in Aquaculture and Fisheries Science 2. Elsevier Scientific Publishing Company, Amsterdam.

Kube, P., M. Cunningham, S. Dominik, S. Parkinson, B. Finn, J. Henshall, R. Bennett, M. Hamilton (2011) Enhancement of the Pacific oyster selective breeding program. CSIRO Marine and Atmosphere Research, *Fisheries Research and Development Corporation and Seafood CRC Final Report,* Project No 2006/2007.

Lacoste, E., N. Gaertner-Mauzoni (2014) Biofouling impact on production and ecosystem functioning: A review for bivalve aquaculture. *Reviews in Aquaculture* **6**, 1-10.

Leavitt, D., M. Griffin (2015) Does the flip bag improve oyster grade and quality? *Journal of Shellfish Research* **34** (2), 585-694.

Leavitt, D., M. Griffin, T. Adam-Cook (2017) Does a flip-bag system produce a better Eastern oyster? *East Coast Shellfish Growers Association (ECSGA) Newsletter* **4**, 2-3.

Li, H., T. Xu, W. Wang, B. Li, J. Chen, G. Sun, Z. Liu, J. Yang (2017) Analysis of heritability, genetic correlation and phenotypic correlation for growth trait in Pacific oyster (*Crassostrea gigas*). *Journal of Fisheries of China* 41 (11), 1680-1686. (*In Chinese with abstract in English*)

Lipton, D., J. Kirkley (1994) A profile of the oyster industry: Northeastern United States. Virginia Institute of Marine Science, University of Maryland.

Lombardi, S.A., G.D. Chon, J.J.W. Lee, H.A. Lane, K.T. Paynter (2013) Shell hardness and compressive strength of the Eastern oyster, *Crassostrea virginica*, and the Asian oyster, *Crassotrea ariakensis*. *The Biological Bulletin* **225**, 175-183.

Loose, S.M., A. Peschel, C. Grebitus (2013) Quantifying effects of convenience and product packaging on consumer preferences and market share of seafood products: The case of oysters. *Food Quality and Preference* **28**, 492-504.

Lunt, J., D.L. Smee (2014) Turbidity influences trophic interactions in estuaries. *Limnology and Oceanography* **59** (6), 2002-2012.

Maccacchero, G.B., J.F. Ferreira, J. Guzenski (2007) Influence of stocking density and culture management on growth and mortality of the mangrove native oyster Crassostrea sp. in southern Brazil. *Biotemas* **20** (3): 47-53.

Mackenzie, C.L. Jr. (1996) History of oystering in the United States and Canada, featuring the eight greatest oyster estuaries. Marine Fisheries Review 58 (4), 1-78.

MacNair, N. (2001) Removal of marine worms from the surface of oyster shell using salt brine. *Aqua Info Aquaculture Notes*, Prince Edward Island, Department of Fisheries, Aquaculture and Rural Development. March 2011.

Mahon, G.A.T. (1983) Selection goals in oyster breeding. *Aquaculture* **33**, 141-148.

Marshall, R.D., A. Dunham (2013) Effects of culture media and stocking density on biofouling, shell shape, growth, and survival of the Pacific oyster (*Crassostrea gigas*) and the Manila clam (*Venerupis philippinarum*) in the suspended culture. *Aquaculture* **406-407**, 68-78.

Matson, S.E. (2010) Development, Evaluation and Application of a Mixed-family Selective Breeding Method for the Pacific Oyster (*Crassostrea gigas*). Dissertation work for the Oregon State University for the Doctor of Philosophy in Animal Science.

Mazón-Suástegui, J.M., K.M. Ruíz-Ruíz, A.Parres-Haro, P.E. Saucedo (2008) Combined effects of diet and stocking density on growth and biochemical composition of spat of the Cortez oyster *Crassostrea corteziensis* at the hatchery. Aquaculture 284, 98-105.

Mehrubeoglu, M., D.K. Smith, S.W. Smith, D.L. Smee, P.A. Simionescu (2013) Investigating oyster shell thickness using three imaging modalities: hyperspectral imaging, thermal imaging and digital photography. *Proceedings of the SPIE- The International Society for Optical Engineering 8870*, Sep. 2013.

Melo, C.M.R., E. Durland, C. Langdon (2016) Improvements in desirable traits of the Pacific oyster, *Crassostrea gigas*, as a result of five generations of selection on the West Coast, USA. *Aquaculture* **460**, 105-115. Messer, K., T. Li, M. Kecinski (2017) Consumer preferences of oyster in Delaware. Fact Sheet of the workshop on Consumer Preferences for Delaware Oysters: An Economic Evaluation of Marketing Messages, Center for Experimental and Applied Economics, Lewes, Feb 2017.

Mizuta, D.D., A. Kasai, K.I. Ishii, H. Yamaguchi, H. Nakata (2014) Effects of artificial upwelling on the environment and reared oyster *Crassostrea gigas* in Omura Bay, Japan. *Bulletin of the Japanese Society of Fisheries Oceanography* **78**, 13-27.

Mizuta, D.D., E.I. Vlachopoulou (2017) Satoumi concept illustrated by sustainable bottom-up initiatives of Japanese Fisheries Cooperative Associations. *Marine Policy* **78**, 143-149.

Murray, T.J., Hudson, K. (2012) Virginia shellfish aquaculture situation and outlook report: results of 2011 Virginia shellfish aquaculture crop reporting survey. Virginia Sea Grant, VSG-12-07, *VIMS Marine Resource Report* **4**, Gloucester, VA. 20 pp.Nel, R., P.S. Coetzee, G. Vanniekerk (1996) The evaluation of 2 treatments to reduce mud worm (*Polydora hoplura claporede*) infestation in commercially reared oysters (*Crassotrea gigas* Thunberg). *Aquaculture* **141**, 31-39.

Nell, J.A. (2001) The history of oyster farming in Australia. *Marine Fisheries Review* **63**, 14-25.

Nell, J.A. (2002) Farming triploid oysters, *Aquaculture* **210** (1-4), 69-88.

Newell, R., V. Kennedy, K. Shaw (2007) Comparative vulnerability to predators, and induced defense responses, of eastern oysters *Crassostrea virginica* and non-native *Crassostrea ariakensis* oysters in Chesapeake Bay. *Marine Biology* **152**, 449–460.

Newkirk, G. (1996) Culture: Genetic improvement. In: Kenedy, V.S., R.E.I. Newell, A.F. Eble (eds.), pp. 75-168. College Park, MD; Maryland Sea Grant College Publication.

Oczkowski, A. J., F. G. Lewis, S.W. Nixon, H. L. Edmiston, R.S. Robinson and J. P. Chanton (2011) Fresh water inflow and oyster productivity in Apalachicola Bay, FL (USA). *Estuarine and Coasts* **34**, 993-1005.

Okuda, K., M. Ohashi (2012) On the studies of recovery and reconstruction of fisheries hit by the Great East Japan Earthquake. *Procedia Technology* **5**, 208-214.

Orton, J.H (1936) Habitat and shell shape in the Portuguese oyster, *Ostrea angulata*. Letters to Editors, *Nature* **138**, 466-467.

Peterson, C.H. (1986) Quantitative allometry of gamete production by *Mercenaria mercenaria* into old age. *Marine Ecology Progress Series* **29**, 93−97

Pinder, L.C.V., T.G. Pottinger, Z. Billinghurst, M.H. Depledge (1999) Endocrine function in aquatic invertebrates and evidence for disruption by environmental pollutants. R&D Technical Report E67. Environmental Agency, Bristol, UK. 147pp.

Pollack, J.B., H.C. Kim, E.K. Morgan, P.A. Montagna (2011) Role of flood disturbance in natural oyster (*Crassotrea virginica*) population maintenance in an estuary in South Texas, USA. *Estuaries and Coasts* **34**, 187-197.

Quayle, D.B. (1988) Pacific oyster culture in British Columbia. Canadian Bulletin of Fisheries and Aquatic Sciences 218, Ottawa.

Robert, R., G. Trut, M. Borel, D. Maurer (1993) Growth, fatness and gross biochemical composition of the Japanese oyster, *Crassostrea gigas*, in Stanway cylinders in the Bay of Arachon, France. *Aquaculture* **110**, 249-261.

Robinson, L.M., J. Lunt, C.D. Marshall, D.L. Smee (2014) Eastern oysters *Crassostrea virginica* deter crab predators by altering their morphology in response to crab cues. *Aquatic Biology* **20**, 111-118.

Roncarati, A., A. Felici, G.E. Magil, N. Bilandzic, P. Melotti (2017) Growth and survival of cupped oysters (*Crassostrea gigas*) during nursery and pregrowing stages in open sea facilities using different stocking densities. *Aquaculture International* **25**, 1777-1785.

Sackton, J. (2013) Reaction to seafood prices expected in 2014: outlook on major market issues and species trends including oysters. *PEI Oyster Conference* 2013, 13-15 Nov., 2013.

Seed, R. (1968) Factors influencing shell shape in the mussel *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom* **48** (3), 561-584.

Soletchnik, P., O. Le Moine, P. Goulletquer, P. Geairon, D. Razet, N. Faury, D. Fouche´, S. Robert (2001) Optimisation of the traditional Pacific cupped oyster *Crassostrea gigas* Thunberg/ culture on the French Atlantic coastline: autumnal fattening in semi-closed ponds. *Aquaculture* **199**, 73-91. Smith, D. (2015) Oyster: A gastronomic history. Abrams, Harry N., Inc. 256 pp.

Song, J., Q. Li, X. Zhong, L. Kong, H. Yu (2017) Genetic diversity and outlier loci detecting of shell color variation in Pacific oysters (*Crassostrea gigas*) by SNP markers. Aquatic Living Resources 30 (10), 1-8.

Suisan Keisai (2014) 日本産に国際競争力を. *The Daily Sui-Kei*, Tokyo, 16278. 27 March 2014. (*In Japanese*)

Taylor, J.T., P.C. Southgate, R.A. Rose (1997) Fouling animals and the effect on the growth of silver lip pearl oysters, *Pinctada maxima* (Jameson) in suspended culture. *Aquaculture* **153**, 31-40.

Thomas, L.L. (2016) The effect of aquaculture gear and tidal zone on the growth and shape of the oyster *Crassostrea virginica* during a “finishing period” in Chesapeak Bay. Master thesis for the Faculty of the Graduate School of the University of Maryland.

Toba, D. (2002) Small-scale oyster farming for pleasure and profit in Washington. Washington Sea Grant program, Seattle, WA.

Toro, J.E., G. F. Newkirk (1991) Response to artificial selection and realized heritability estimate for shell height in the Chilean oyster *Ostrea chilensis*. *Aquatic Living Resources* **4**, 101-108.

Wada, K.T. (1986) Genetic selection for shell traits in the Japanese pearl oyster, *Pinctada fucata martensii*. *Aquaculture* **57**, 171-176.

Walton, W.C., J.E. Davis, G.I. Chaplin, F.S. Rikard, T.R. Hanson, P.J. Waters, D.L. Swann (2012) Timely information: Off-bottom oyster farming. Fisheries and Aquaculture Series. Alabama Cooperative Extension System.

Walton, W.C., F.S. Rikard, G.I. Chaplin, J.E. Davis, C.R. Arias, J.E. Supan (2013) Effects of ploidy and gear on the performance of cultured oysters *Crassostrea virginica*: survival, growth, shape, condition index and *Vibrio* abundances. *Aquaculture* **414-415**, 260-266.

Wan, S., Q. Li, T. Liu, H. Yu, L. Kong (2017) Heritability estimates for shell color-related traits in the golden shell strain of Pacific oyster (*Crassostrea gigas*) using a molecular pedigree. *Aquaculture* **476**, 65-71.

Wang, Q., Q. Li, L. Kong, R. Yu (2012) Response to selection for fast growth in the second generation of Pacific oyster (*Crassostrea gigas*). *Journal of Ocean University of China* **11**, 413-418.

Ward, R.D., P.A. Thompson, S.A. Appleyard, A.A. Swan, P.D. Kube (2005) Sustainable genetic improvement of Pacific oysters in Tasmania and South Australia. FRDC Final Report Project No. 2000/206. CSIRO Marine and Atmospheric Research, Hobart. Australia.

Wheaton, F., S. Hall (2007) Research needs for automated oyster shucking. *Aquacultural Engineering* **37**, 67-72.

Xiong, G., D.J. Lee, K.R. Moon, R.M. Lane (2010) Shape similarity measure using turn angle cross-correlation for oyster quality evaluation. *Journal of Food Engineering* 100, 178-186.Xu, L., Q. Li, H. Yu, L. Kong (2017) Estimates of heritability for growth and shell color traits and their genetic correlations in the black shell strain of Pacific oyster *Crassostrea gigas*. *Marine Biotechnology* **19**, 421-429.

Zhang, Y., Y. Qin, Y. Zang, J. Li, S. Xiao, Z. Xiang, H. Ma, Z. Yu (2016) Population selection for growth in two strains of the Kumamoto oyster *Crassostrea sikamea*. *Journal of Fisheries Science of China* **23** (4), 882-889. (*In Chinese with abstract in English*)

Table 1: Common employed methods for improvement of oyster shell traits.

Oyster shell trait	**Applicable method**	**Selected reerences**
Overall shape (no deformities)	Single seeds	Toba (2002); Kato-Yoshinaga et al. (2014)
Length	Selective breeding	Wada (1986); Ward et al. (2005); Kube et al. (2011); Kong et al. (2015)
	Off-bottom culture	Bishop & Peterson (2006); Dunham & Marshall (2012)
Width	Selective breeding	Wada (1986); Ward et al. (2005); Kube et al. (2011); Kong et al. (2015)
	Low stocking density	Davis (2013); Marshall & Dunham (2013)
Depth	Low stock density	Davis (2013); Marshall & Dunham (2013)
	Tumbling (mechanical, tidal)	Cheney (2010); Leavitt et al. (2017)
	Selective breeding	Wada (1986); Kube et al. (2011); Walton et al. (2013); Kong et al. (2015)
	Ploidy	Matson (2010); Dégremont et al. (2012); Walton et al. (2013)
Cleanliness	Culture media	Dunham & Marshall (2012); Marshall & Dunham (2013)

(biofouling containment)	Freshwater baths	Quayle (1988); Nel et al. (1996)
	Salt brine baths	MacNair (2001)
	Manual cleaning and scraping	Quayle, 1988; Toba (2002)
	Air exposure	Quayle (1988); Handley & Bergquist (1997)
Colour	Selective breeding	Ward et al. (2005); Evans et al. (2009); Kang et al. (2013); Song et al. (2017)
Thickness (hardening)	Moderate energy environment (intertidal areas; wave exposure)	Orton, 1936; Toba (2002); Thomas (2016)
	Tumbling (mechanical, tidal)	Robert et al. (1993); Leavitt et al. (2017)

Figure Legends

Figure 1: Shell shape in reared oysters: identification of shell common measurements shown in oyster frontal and side views, respectively.

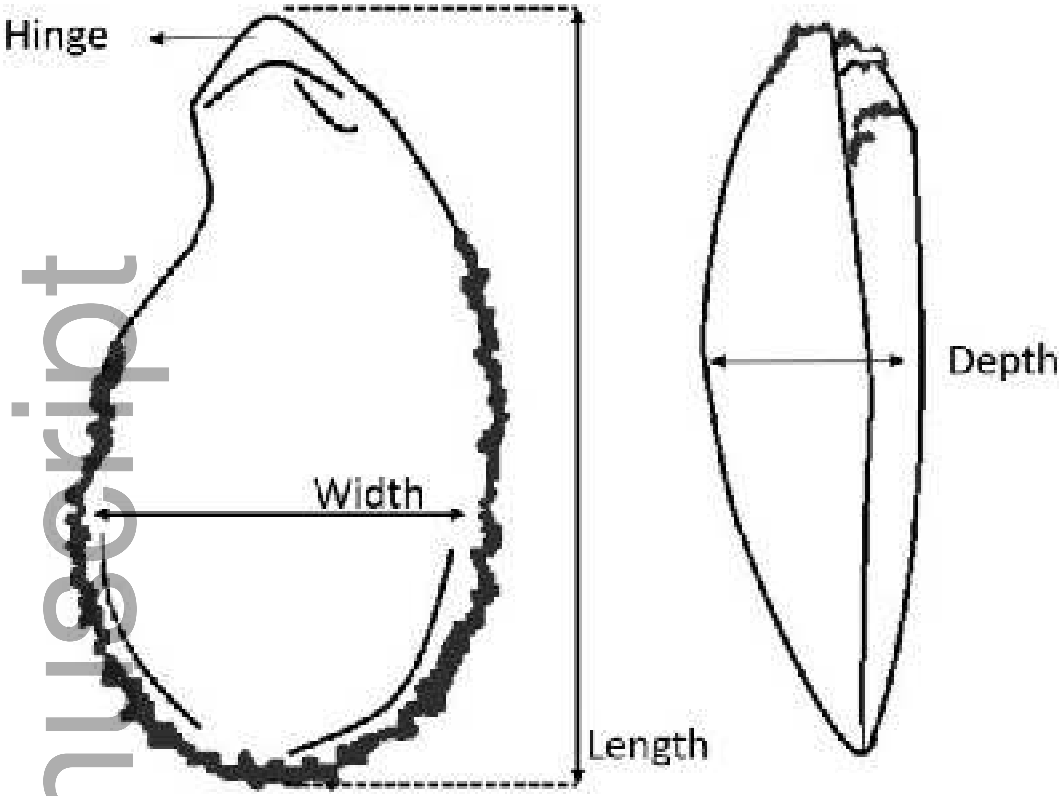
Figure 2: Oyster grading scheme employed in Canada for *Crassostrea virginica* based on shell length and width (Doiron, 2008; reproduction authorized by the New Brunswick Department of Agriculture, Aquaculture and Fisheries).

Figure 3: Unappealing shell blisters (indicated by arrows; left) caused by the oyster-worm *Polydora spp*. (indicated by the arrow; right), that can often leave the holes it bores in the shell and “frighten” shellfish eaters.

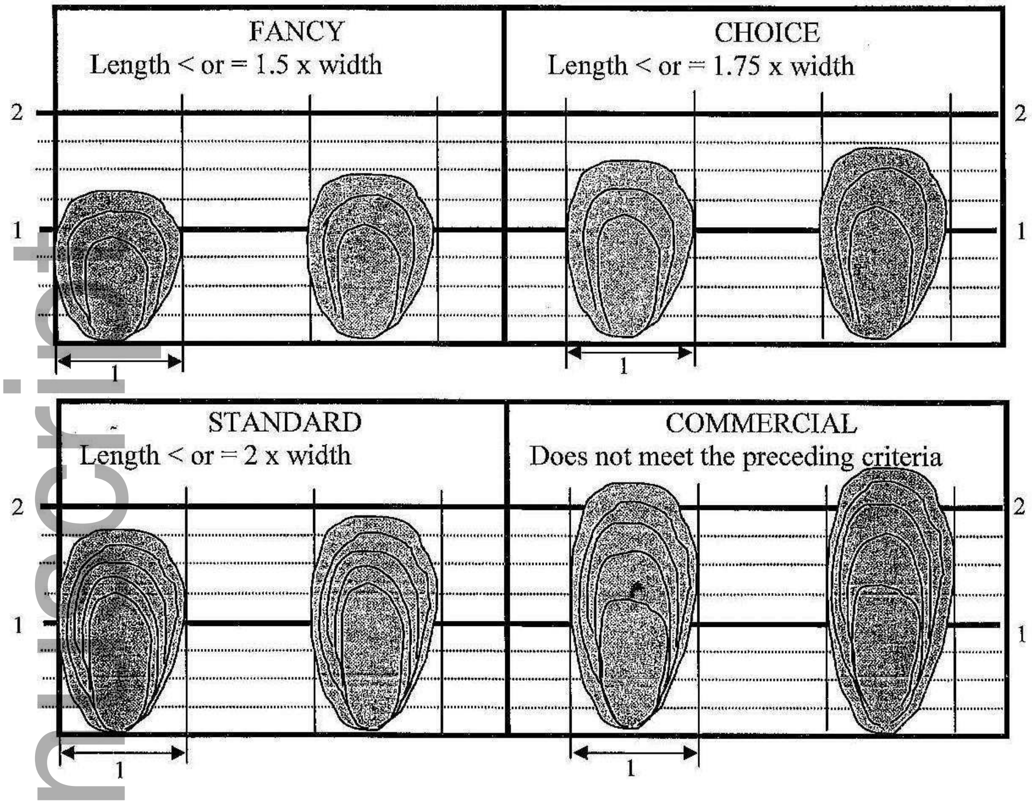
Figure 4: Design of an oyster culture apparatus that allows for temporary air-exposure *in situ* targeting biofouling control (Authorized use of photos of the OysterGro® technology by the Bouctouche Bay Industries).

Figure 5: Examples of husbandry practices and yields in oyster farming: **a)** Oysters clusters resulting from the grow-out using the spat-on-shell method in Japan; and the contrasting **b)** individual oysters resulting from single seeds that are grown individually glued to ropes in New Zealand (Authorized use of photo from the TOPSoyster

company); **c)** Floating bags for tumbling in vertical position during low tide d) Oyster baskets that shake with water movement and facilitate tumbling (Authorized use of photo from Seapa company). Bags would horizontally float during high tide (Authorized use of photo from the Taylor Shellfish Farms company; photo credit: Kristian Marsden).

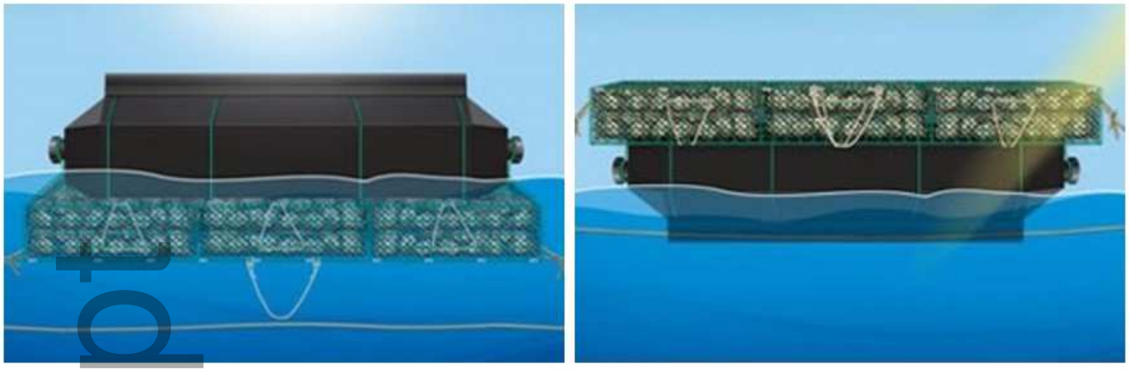


raq_12247_f1.jpg

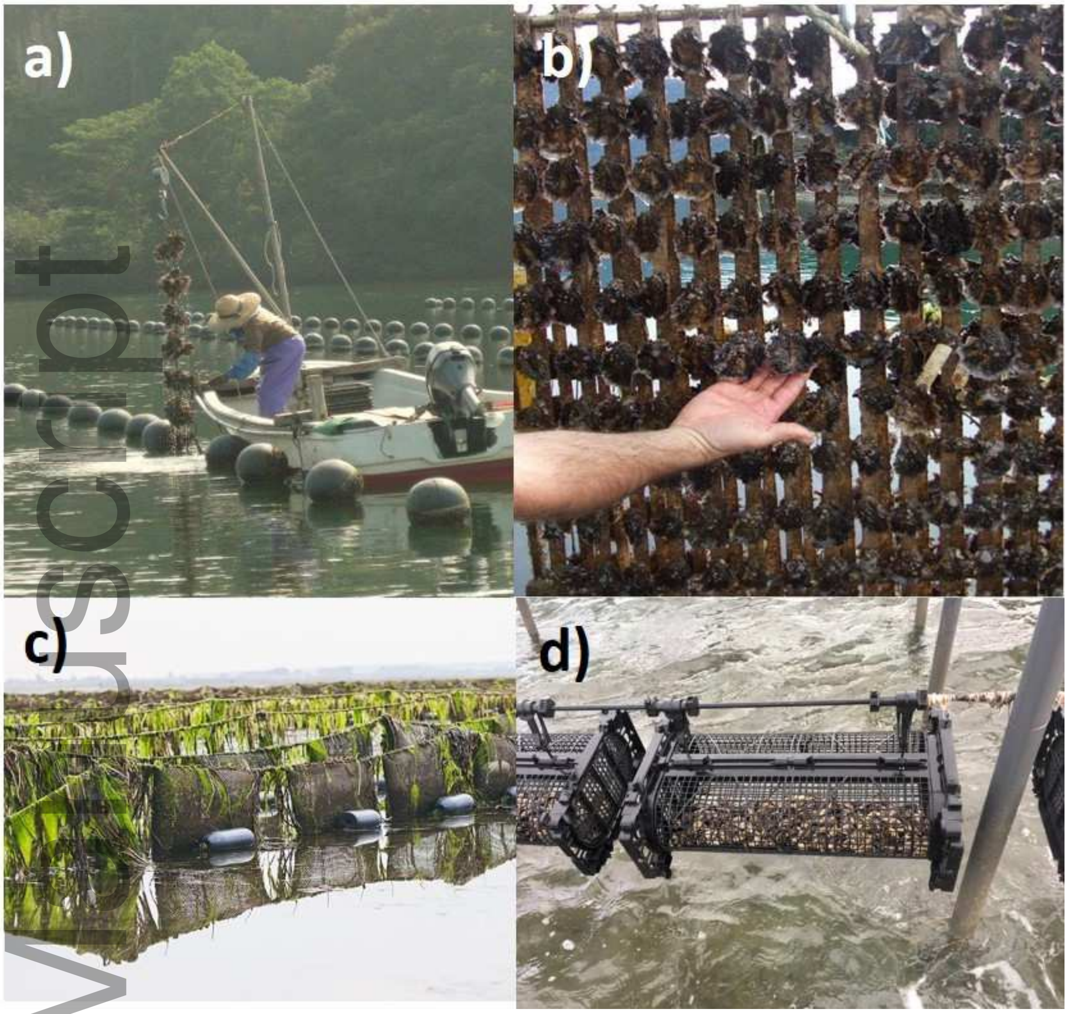


raq_12247_f2.jpg

raq_12247_f3.jpg



raq_12247_f4.jpg



raq_12247_f5.jpg