Aquat. Living Resour. 24, 227–236 (2011)

DOI: 10.1051/alr/2011144
www.alr-journal.org

Aquatic Living Resources

Review and perspectives of physiological mechanisms underlying genetically-based resistance of the Pacific oyster *Crassostrea gigas* to summer mortality

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Received 1st February 2011; Accepted 18 July 2011

Abstract – French oyster farming has been subject to severe mortalities during the summer months. Results from the research program "Morest", which ran from 2000 to 2006 and examined the possible causes of these mortalities, led to the construction of a model to explain the interaction between environmental factors, oyster physiology and different opportunistic pathogens underlying oyster summer mortality. Temperature, food, reproduction and stress were the main factors required for oyster mortality. Genetically-based resistance ("R" oysters) or susceptibility ("S" oysters) to summer mortality was revealed by divergent selection. Building on these results, a literature search was made in 2007 on the molecular origin of genetic resistance to such a complex mortality risk. The objectives were to lay a foundation for the preparation and orientation of future research directions and to improve understanding of the underlying physiological mechanism leading to summer mortality. Three years later, the resulting conceptual analysis reported here was presented as an introductory lecture to Physiomar 2010, a conference where many new results contributing to this research field were also reported. The literature review highlighted two major review articles: the first dealing with nutrition and reproduction (Schneider 2004), the second with reproduction, temperature, oxidative stress and mortality (Heineinger 2002). The effect of nutrition level on energy orientation to growth or reproduction is controlled by endocrine factors. Among these, neuropeptide Y (NPY), ghrelin and leptin neuropeptides appeared to be potential candidates involved in germ-soma orientation in relation to trophic conditions. Depending on reproductive effort and temperature, a metabolic stress resulting from the germ-soma conflict can appear, characterized by mitochondrial reactive oxygen species (ROS) production. Such an excess of ROS induces perturbations in mitochondrial activity leading to cell death. Many organisms, such as annual plants or the Pacific salmon, do not survive their first reproduction. In contrast, others increase stress resistance by selection of antioxidant processes (superoxide dismutase SOD, catalase, etc.) through evolution, and survive first reproduction. A similar difference was observed in the comparison made between R and S oysters, which differed in ROS production, SOD and catalase levels. Such factors controlling reproduction and ROS detoxification processes could therefore provide new markers for selection of oysters with better resistance to non-specific pathogens, complementing other classic selection approaches against specific pathogens or for improved immunity. This antioxidant defence mechanism is found in many organisms including vertebrates and in some invertebrates, including oysters. Its role needs to be considered in pathology events involving other aquaculture species and it may also contribute to explaining the increase in marine pathologies under anthropogenic environmental changes.

Key words: Immunity / Antibacterial activity / Host defence / Environmental stress / *Crassostrea gigas*

1 Introduction

Summer mortality of the Pacific oyster *Crassostrea gigas* is the result of complex interactions between oysters, their environment and opportunistic pathogens. The relationship of these factors is shown in Figure 1, as found by the French Program termed Morest (*Mortalité estivale*) performed from 2000 to 2006 (Samain et al. 2007; Samain and McCombie 2008, summarized in Samain (2008). Temperature is the foremost important parameter in this model; it opens a window of risk for oysters once the critical threshold of 19 °C has been passed. High trophic conditions induce large reproductive effort which increases the mortality risk, but temperature and reproductive effort alone are not sufficient to cause summer mortality, a stress is necessary. In most cases of summer mortality a sediment-related stress has also been detected, due to organic matter mineralisation in anoxic conditions leading to hydrogen sulphide release at the sediment surface. Three interesting features characterize this interaction process: (1) all the parameters are necessary; the exclusion of one, e.g., food level, temperature or stress, leads to a much lower mortality risk;

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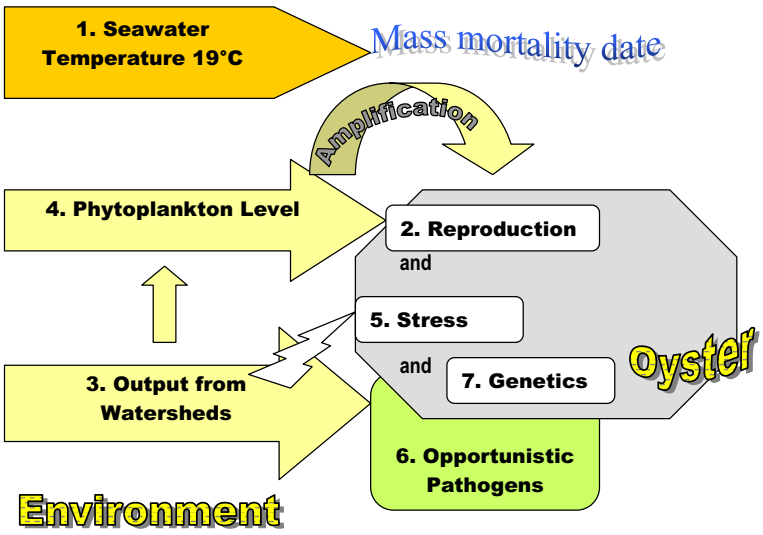

Fig. 1. Hierarchical interaction model: a combination of essential factors leading to *Crassostrea gigas* oyster summer mortality risk. (1) a critical threshold at 19 °C temperature opens a window of risk, thus predicting a possible date of mass mortality, if (2) a high reproductive activity is induced by (4) high trophic conditions resulting from (3) intensive annual rains that provide nutrients and organic matter from watersheds. Lastly, a stress is necessary (most frequently from organic matter degradation in the sediment). Oyster defences are differently affected depending on (6) their genetics, favouring (7) infection of the more susceptible oysters by opportunistic pathogens (after Samain et al. 2008, *Morest* project).

(2) different pathogens, such as bacteria (*Vibrio splendidus, V. aestuarianus*) and viruses: ostreid herpesvirus 1 (OsHV-1, and microvar OsHV-1 Renault et al. 2011), infect the oysters; (3) very significant genetically-based resistance ("R" oysters) or susceptibility ("S" oysters) to summer mortality is possible, as revealed by divergent selection experiments. At the end of this program, the nature of the genetic mechanism that could form the basis of such a complex interaction and non-specific resistance remained a major question. This initiated a literature search to evaluate the relevance of the Morest results with regard to broader scientific knowledge, which provided interesting results from the living world as a whole and a conceptual analysis leading to some directions that will guide new research. In 2010, this conceptual analysis was presented as an introductory lecture for the Physiomar 2010 meeting, Québec. The aim of the present paper is to evaluate the relevance of its scientific approach by looking at newly published results and relevant articles.

The literature search was performed to look for possible candidate genes underlying such a complex interaction. The seven keywords used were Temperature, Nutrition, Reproduction, Energy, Stress, Immunity and Mortality. Among the many papers selected by the search process were two very complementary major review publications: "Energy balance and reproduction" (Schneider 2004) and "Aging is a deprivation syndrome driven by a germ-soma conflict" (Heineinger 2002). The 2004 Schneider review concerns mammals and describes the complex network of factors from the peripheral or central nervous systems, which are involved in the control of feeding and energy allocation. According to this review, the availability of oxidizable products of glucose and free fatty acids (FFA) from nutrition is detected and, according to these levels, feeding activity is controlled and energy balance orientated to either storage or reproduction. In low trophic conditions, inhibition of the hypothalamic-pituitary-gonadal system (HPG) and reproduction lead to an energy storage priority for survival and to an activation of feeding behaviour. Inversely, in high trophic conditions, stimulation of HPG induces energy allocation to reproduction and inhibition of eating. Among the numerous regulatory factors involved, three neuropeptides appeared to be particularly important: neuropeptide Y (NPY) and its receptors from the HPG, ghrelin and leptin (fom peripheral neurones). NPY and ghrelin increase at low food levels. Both these factors contribute to inhibition of reproduction and activation of feeding behaviour, allowing energy storage. Inversely, leptin levels increase with energy storage. High leptin levels lead to decreases in NPY and ghrelin, and the activation of reproduction process and inhibition of feeding activity (Fig. 2, after Schneider 2004). These results reveal important relationships between food availability, feeding behaviour, energy storage or reproduction. The three neuropeptides are also found in some invertebrates, e.g., Tensen et al. (1998) cloned the first NPY receptor from the mollusc gastropod *Lymnaea stagnalis*. According to de Jong-Brink et al. (2001), who compared the molecular structure of NPY in invertebrates, only molluscan and arthropod NPY are synthesized from a prohormone similar to vertebrate NPY and should be considered as true invertebrate homologs of vertebrate NPY. In *Lymnaea stagnalis*, NPY plays a key role in regulating energy flows in relation to NPY receptor Y1. It stops the main energy consuming processes, reproduction and growth, but has no effect on food intake. It is not known whether this situation is specific to *Lymnaea stagnalis*, as data on biological functions of NPY in other molluscs or invertebrate species are lacking. In Crustacea, Kiris et al. (2004) reported the effect of NPY on food intake and growth of penaeid shrimps. In fish, NPY has been detected in the rainbow trout *Oncorhynchus mykiss* (Larsson 2006) and catfish *Clarias*

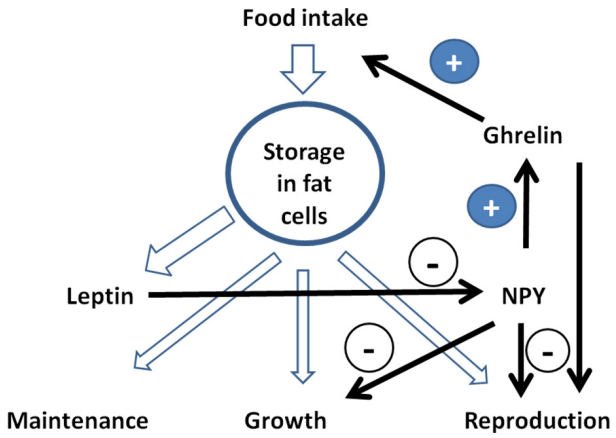

Fig. 2. Schematic presentation of the positive and negative control of neuropeptide Y (NPY), ghrelin and leptin on food intake, energy storage in fat cells, maintenance, growth and reproduction, according to Schneider (2004).

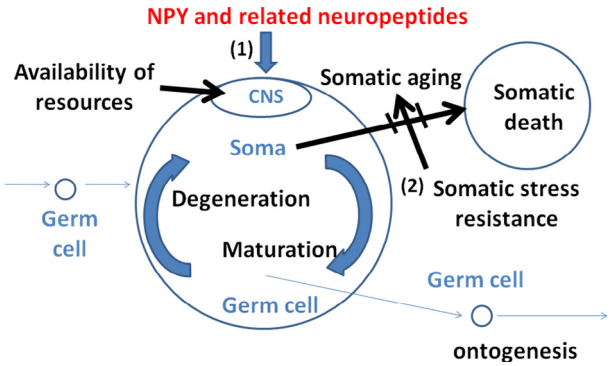

Fig. 3. Energy balance and reproduction (Schneider 2004) and germ-soma conflict theory (Heininger 2002): positive availability of resources induces a hormonally controlled orientation of energy balance towards reproduction. Among hormonal cues (1) NPY and related neuropeptides are putative effectors acting on the central nervous system (CNS). Under high trophic conditions, when stored nutrients are available, energy balance is orientated to germ cell maturation. In return, this will induce an oxidative stress detrimental to somatic cells, leading either to somatic death or, if (2) stress resistance mechanisms based on antioxidant capacities, super oxide dismutase (SOD) and catalase have been selected through evolution, to somatic aging.

batrachus (Gaikwad et al. 2004; Singru et al. 2008). Ghrelin was also found in *Drosophila* (Hewes et al. 2001), but no information is available on its function in this genus. The gene for ghrelin has been recently identified in several fish species. As in mammals, ghrelin mRNA, is mainly expressed in the gut of fish and is involved in the regulation of a number of physiological functions, including the stimulation of food intake (Unniappan and Peter 2005). A leptin-like factor is produced by glycogen storage cells in *Lymnaea stagnalis*, which inhibits food intake (de Jong-Brink 2001), and in the Chinese crab *Eriocheir sinensis*, where the results suggest that leptin may play a critical role in the regulation of reproductive maturity (Jiang et al. 2010). Based on electrophoretic mobility, immunoreactivity, response to fasting and correlation with adiposity, Johnson et al. (2000) reported the first evidence for leptin expression in ectotherms in a study on six species of fishes. Although these factors and their function appear relatively conserved through evolution, more investigations are necessary to improve it.

In the second review, "Evolution and reproductive strategy, the germ-soma conflict" by Heininger (2002), it is proposed that living organisms have had to adapt to a shortage of resources during their evolution, depending on environmental conditions. In unicellular organisms, exponential growth of bacteria is limited by trophic conditions. "Deprivation-induced cell death and differentiation into germ cell-like spores can be regarded as archaic reproduction events which were fuelled by the remains of the fratricided cells of the apoptotic fruiting body". This mechanism has become fixed in multicellular organisms, where somatic death would be triggered by germ cells in conditions of finite resources. As shown by Schneider (2004) in a schematic representation (Fig. 3), when trophic and stored resources increase, the neuroendocrine system, particularly those components that control energy balance and reproduction, including neuropeptides like NPY, ghrelin and leptin, will orchestrate the regulation of energy flow towards reproduction. However, during evolution, germ cells in many organisms will negatively affect longevity of somatic cells. In invertebrates such as *Caenorhabditis elegans* (Nematode) and *Drosophila*, germ cells affect somatic longevity via steroids or sex hormone-like signals (Heininger 2002). The gonadal hormone-mediated deterioration of the somatic trophic milieu elicits a systemic and cellular metabolic stress response (Heininger 1999). Mitochondria have been shown to be a central key affecting lifespan (Kirkwood and Kowald 1997). The major energy source in mitochondria is nutrient oxidation through the Krebs cycle and respiratory chain, which consumes O_2 and produces ATP, CO_2 and by-products like reactive oxygen species (ROS). Increased metabolic rate and respiration activity is associated with a higher mitochondrial ROS generation and lipid peroxidation potential (Sohal and Weindruch 1996; Gabbita et al. 1997; Barja 2000). Such an increase in metabolic rate is observed when reproductive development starts. Moreover, in ectotherms, these metabolic activity and energy demand are also increased with spring temperature, leading to an increase in mitochondrial ROS production. Moreover, the metabolic stress resulting from the germ-soma conflict is also characterized by oxidative stress, which is the effector of many other biotic and abiotic stressors (Heininger 2001). Such an excess of ROS induces perturbations in mitochondrial activity and membranes, mitochondrial DNA mutations and energetic deficiency, leading to cell death. According to Heininger (2002), this can be observed through evolution, from nematodes to mammals. Aging (or survival), as opposed to somatic death, is a mechanism that counteracts the negative effect of germcells. In a more general way, stress tolerance is also a primary modulator of aging processes. ROS detoxification processes would play a role in mitochondrial integrity, cell survival and longevity, protecting mitochondrial structure and activity. Some enzymes, such as superoxide dismutase (SOD) and catalase, play an important role in ROS detoxification. Other enzymes, such as glutathion peroxidase, glutathione reductase and superoxide

reductase, or molecules like CoQ (coenzyme Q), metallothioneins (MT), melatonin, vitamin C, vitamin E, carotenoids, flavonoids, terpens and polyphenols, and some metals, such as Se, Fe, Zn, Mn and Cu, can also destroy reactive oxygen species. In his review, Heininger (2002) noted that an increase in antioxidant defences and stress proteins were cellular markers of extended longevity and reduced reproductive activity. ROS detoxification processes protect mitochondrial integrity and consequently influence lifespan: depending on organisms, some species invest highly in reproductive activity, but die after their first reproduction (annual plants, Pacific salmon and octopus). This is known as the "r" strategy (MacArthur and Wilson 1967). Other species invest less in reproduction and more in oxidative stress resistance ("K" strategy). They survive after their first reproduction, allowing for parental care and/or repeated reproductive cycles. One main difference between "r" and "K" strategies is the difference in ROS detoxification capacities by selection of antioxidant processes (SOD, catalase, etc.) through evolution.

In mollusc species, large differences in longevity are observed. The ocean quahog *Arctica islandica* is the longest-lived of all bivalve and mollusc species on the earth, living close to 400 years. Low metabolic rate and superoxide dismutase activities maintained at high levels throughout life may explain the long lifespan of this species (Abele et al. 2008). In contrast, high proliferation rates in tissues of the short-lived scallop *Aequipecten opercularis* (lifespan: 2–4 years) are in line with high energy throughput and reduced investment in antioxidant defence mechanisms (Strahl and Abele 2010; Guerra et al., pers. comm.). It has indeed been remarked that oxidative stress and longevity are inversely related when disparate species groups, like bivalves and birds, are considered (Buttemer et al. 2010).

From the present literature search, it was concluded that nutrition, reproductive effort, stress and survival or mortality are linked in a logical way. Nutrition controls energy balance and reproduction, resulting in an increase in energy demand that is amplified by temperature (in poikilotherms). Reproductive activity is a major pacemaker of aging and death: increases in reproduction and temperature induce a germ-soma conflict (metabolic somatic stress) and an increase in mitochondrial ROS production. Other environmental stresses also amplify ROS production. Depending on whether an organism has an active or weak ROS detoxification capacity, mitochondrial activity will be protected or destroyed under such stress, leading to conditions favouring survival or death, respectively due to differences in energetic and immune capacity and resulting varying vulnerability to infection by pathogens. The important roles of mitochondrial activity and ROS detoxification processes in lifespan control should be underlined; this mechanism has been highly conserved through evolution.

2 *Crassostrea gigas* summer mortality in literature

2.1 A multidisciplinary research program: MOREST results

Experimental studies on oyster summer mortality (Lambert et al. 2008) have shown that high trophic conditions induce higher oyster reproductive effort and susceptibility to experimental infections, leading to higher mortality rates than low trophic conditions. Oyster respiration rate also increases correlatively with reproductive effort and is exponentially amplified by temperatures around 19 °C, indicating a large energy demand at high food level. These results fit well with results reported in the literature, showing a control of energy balance and reproduction by nutrient availability, and the important increase in respiration with food level and temperature. It should be considered whether the highest mortality rates observed in high trophic conditions are related to a germ-soma conflict and reduction of life span according to Heininger's theory (2002). Resistant (R) and susceptible (S) lines of oysters were selected starting from natural populations (Dégremont 2003; Dégremont et al. 2007; Boudry et al. 2008). In addition to lower survival, physiological comparison revealed that S oysters had higher reproductive effort than R oysters and showed multiple partial spawnings (Delaporte et al. 2007; Samain et al. 2007; Huvet et al. 2008). When environmental temperature and, hence, metabolic rate increased in spring, production of ROS increased in S oysters over the two months preceding the mortality period, exceeding levels recorded in R oysters. S oysters also died more frequently than R oysters at 19 °C. The difference in ROS levels between R and S would correspond to the oxidative stress resulting from germ-soma conflict and other abiotic stressors. Preliminary results showed that superoxide dismutase (SOD) and catalase activity were higher in R oysters, and heat shock protein (HSP70) was higher in S oysters (Samain et al. 2007). These first results fit quite well with the r and K strategy model proposed for different species (MacArthur and Wilson 1967). Here, however, these strategies are clearly shown within natural populations of *C. gigas*. Jeschke et al. (2008) confirmed that such life histories showed patterns both within and among species. This conceptual analysis needed to be investigated in greater depth, so cDNA libraries were developed in the laboratory to allow a genomic approach.

2.2 New gene expression results

Huvet et al. (2010) showed that high reproductive effort was a trait associated with susceptibility to *C. gigas* summer mortality. Fleury et al. (2009, 2010) provided extended results using oyster microarrays and real-time polymerase chain reaction (PCR). These studies compared differential mRNA expression of R and S oysters in natural conditions during a three month period including a mortality event. As early as two months before the mortality peak, R oysters showed significant over expression of two clusters of genes coding for (1) energy balance and reproduction control, like NPYr, transforming growth factor *beta* (TGFβ, reproduction, germinal cell maturation) and dopamine receptor (energy balance/reproduction); and (2) coding for ROS detoxification (SOD, catalase), ROS control (cytochrome *c* oxidase, COX), marker of mitochondrial electron transport activity (group 4), essential for limiting ROS production, and acylcarnitine carrier protein (ACP), for alternative energy production through lipid oxidation limiting oxidative stress. These latter results confirm that the control of energy balance is different between R and S oysters and that

genes involved in detoxification processes are more expressed in R oysters than in S ones. Lastly, only S oysters were seen to have an over expression of HSP70, suggesting oxidative stress resulting from mitochondrial respiratory and SOD/catalase deficiencies, as observed over the aging process in *Drosophila* (Kuzmin et al. 2004). This could be interpreted as an ultimate attempt by S oysters to react to the metabolic stress imposed by germ-cells on the soma when detoxification processes are lacking. An over expression of immune genes in R oysters was also observed, which was probably in response to pathogen infection just before mortality, a hypothesis confirmed at the last analysis date that fell during a mortality event (Fleury and Huvet 2011).

These results confirm, as theoretically expected from the literature, that differences in reproductive activity, ROS production and mortality rate between S and R oysters can be compared to the "r" and "K" strategy described above.

S oysters would have an "r-like" strategy (a single reproduction), with a higher reproductive activity than R oysters and no efficient ROS protection, leading to a high mortality when stress and temperature increase during the reproductive period. R oysters would have a "K-like" strategy, with lower reproductive activity than S oysters, better ROS protection and consequent better resistance to stress, leading to lower mortality.

This conceptual mechanism can be considered with regard to a wealth of existing literature that deals with the role of energy limitation and trade-offs in a variety of organisms, e.g., Warren (1967), Bayne (1983), Widdows (1988), Mori (1979), Kooijman (2000) and Saraiva et al. (2011). During the Morest program, this aspect was taken into account and the Dynamic Energy Budget (DEB) model from Kooijman was adapted to oysters (Pouvreau et al. 2006; Samain and McCombie 2008). Although interesting results were obtained and taken into consideration, energy deficiency did not explain all the situations observed, especially in very rich trophic areas. Summer mortality was observed in these areas even when oysters had a high glycogen content, leading to the conclusion that all underlying physiological mechanisms were not taken into account in the ecophysiological model (Grangeré et al. 2009). However, even though ecophysiological models do not fit observations exactly, there are links between energy and survival in our conceptual hypothesis: metabolic rates increase exponentially with food ingestion and temperature, inducing related rate of ROS production by the mitochondria. The food metabolites and hormones induce energetic orientation to germinal cells leading to deprivation of somatic cells inducing detrimental oxidative stress to mitochondria and damage to cellular energetic production. Defence mechanisms between R and S oysters appeared different (A. Huvet et al. 2010; Fleury and Huvet 2011): after the important ROS increase observed in haemolymph, S oysters only expressed a stress HSP70 marker and then died, while R oysters expressed less HSP70 but numerous putative defence genes and survived better than S oysters. This result can be interpreted as a detrimental effect of ROS increase on the further expression of defence genes in S oysters, possibly caused by the detrimental effect of oxygen radicals on mitochondria, decreasing the energetic source they represent.

3 Implications for future research strategy

Two strategies for improving survival are offered according to the proposed scheme (Fig. 3): (1) at the level of energy balance control, to decrease ROS production by reduction of reproductive effort and reduction of stress (hereafter named strategy 1); or (2) by the control of the ROS detoxification process (hereafter named strategy 2).

In strategy 1, energy balance orientation towards reproduction depends on reserve storage, food level and genetic neurohormonal control. In natural conditions, reproduction orientation is under control of food availability and temperature. In *C. gigas* oysters in the natural environment, the first reproduction of offspring from year *n* can be observed in spring of the following year or the one after (at 18 months of age), depending on the trophic conditions and temperature. However, full sexual maturations were observed as early as 3-months-old and in 10 mm spat, when these were reared in an intensive nursery during the Morest program. This early maturity means this spat can be susceptible to mortality at this small size, as soon as high food levels orientate the energy balance towards germinal cell development.

It is possible to decrease reproductive activity through husbandry management techniques, like decreasing food level or food access for young spat. This process, called caloric restriction in many other species (Heininger 2002), is known in yeast (Lin et al. 2000), nematodes (Hosono et al. 1989), rodents (Weindruch and Walford 1988; Masoro 1998) and in primates and humans (Wanagat and Weindruch 2000). At the cellular level, caloric restriction inhibits the aging-related increase of mitochondrial ROS release and level of oxidative stress, and attenuates the decrease of antioxidant defences (Rao et al. 1990; Gabitta et al. 1997; Risby et al. 1999, etc.). In oysters, the empirical practice known as "stunting" or "hardening" in Japan, China, Korea, USA and New Zealand oyster production (Terashima et al. 1978; Katayama et al. 1979; Handley 1997) consists mainly of a reduction of feeding time by placing young spat on the upper part of the shore in tidal areas and leads to better survival, as observed in the Morest project. Another way is to reduce energy demand by rearing oysters during the critical reproductive period in areas with water temperature around 15 °C, such as in the North Brittany Aber areas (Iroise Sea thermal from Ushant, France). Lastly, better environmental care at this stage can reduce possible stressor effects (rearing far from the sediment). Selection of oysters demonstrating a K strategy should be pursued based on genes involved in energy balance control. These genes should be chosen among those that are differentially expressed between R and S oysters in relation to the reproductive process, such as NPY and its receptors (L. Bigot, pers. comm.), transforming growth factor *beta* (TGFβ, Fleury 2008) and hexokinase, but also other theoretically-related candidates such as ghrelin, leptin, insulin/IGF after A. Jouaux et al.[(1)], vasa (Fabioux 2004, 2009) and adenosine 5'-monophosphate (AMP)–activated protein kinase (AMPK) after E. Guévélou et al.[(1)]

Strategy 2 concerns the complex reactive oxygen species (ROS) process. Numerous experiments performed on

[(1)] Communications presented at Physiomar 2010, Québec, November 2010

vertebrates and invertebrates show a detrimental effect of ROS on lifespan, which contrasts with the clear positive effect of detoxification enzymes like SOD and catalase. Again, it would be interesting to focus on selection of genes that are differentially expressed between R and S, as they confirm the important role of the enzymes SOD and catalase for survival. Other similar ROS detoxification mechanisms, like metallothionein (MT) or glutathion peroxidase (GP), could also probably be of interest. Research priorities in this domain are necessary, and the expansion of research in these directions has been illustrated by a number of communications presented at Physiomar 2010, including some published in the present issue, and by other recent articles: Franco et al. (2011), Llera-Herrera et al.(1), Kellner et al.(1), Jouaux et al. (2011) and Guévélou et al.(1) in the field of cellular and molecular aspects of reproduction, Beguel et al.(1) on oxidative stress and reproduction, Kurochkin et al.(1), Guderley et al. (2011) and Dudognon et al. (1) on mitochondrial activity and ROS production, Donaghy et al.(1), Pichaud et al.(1) and Kurochkin et al.(1) on oxidative pathways, Munro and Blier(1), Fleury et al. (2010), Strahl et al. (2010) and Guerra et al.(1) on oxidative stress resistance, longevity and survival, and Sussarellu et al. (2010) and Camara and Langdon (1) on stress related genes.

4 Is the increased mortality observed in *C. gigas* since 2008 due to a different mechanism?

From 2008 onwards, a higher mortality rate was observed in juveniles during their first year than had been observed in previous summers. It is difficult to provide a fully documented opinion on this phenomenon, as many observations are not yet published. In my opinion, a number of different hypotheses can be put forward:

- Analysis of risk assessment in early spring 2008 showed that there was a major mortality risk in 2008: after a long period of rainfall deficiency from 2003 to 2006, high levels of rain were measured in fall 2007 and early spring 2008. A large quantity of nutrients stored in the fields over these preceding three years then entered the marine ecosystem from the watersheds. This nutrient increase would have boosted the intensity of the phytoplankton bloom, and spat feeding activity probably accelerated reproductive metabolic orientation and, hence, oyster susceptibility to stress and infection. This could be one explanation of the high juvenile mortality observed in 2008. Thereafter, another major factor was identified: faced with such high mortality, growers made many transfers of oysters between production areas and this favoured the spread of pathogens. Moreover, a large increase in spat density within rearing bags increased transmission, mortality and pathogen concentration everywhere. This led to a huge epizooty event with an increase in the ostreid herpes virus OsHV-1 microvar mutant (Segarra et al. 2010) and *Vibrio splendidus*. These cumulated events could have completely modified the conditions of the Morest interaction model due to the dominance of one of the critical factors: the high pathogen prevalence. Today, this high prevalence is the basis of the hypothesis for the enhanced mortality, as a very high transmission rate was observed whatever the oyster origin. It is very important now to examine oysters from the epizooty origin each year to search for a putative initial group that are more susceptible. Such an approach is possible because different mortality dynamics are observed for oysters from different origins when these are reared separately.
- Another hypothesis is an increase in pathogenicity of the ostreid herpes virus OsHV-1 microvar mutant, which is under study. This could also explain the high transmission rate, but *Vibrio splendidus* was also detected. Among the *V. splendidus* strains found, some virulent ones are known to kill oysters and could also be involved (De Decker et al. 2011).
- Another important change is that triploid (3n) oysters resisted summer mortality until 2006 (Gagnaire et al. 2006) but, over the last three years, they have been as susceptible as diploids. Under natural conditions, previous studies reported variable results on the relative resistance of triploid oysters to summer mortality according to the technology used to produce them - 3n cytochalasin or tetraploid technologies (Allen and Downing 1986; Guo et al. 1996). Two types of gametogenic paths were described in triploid oysters by Jouaux et al. (2010). Duchemin et al. (2007) found a minimum phagocytic index, as in diploids, during the triploid "spawning period", and noticed that triploids spawned a month earlier than diploids. De Decker et al. (2011) reported a period of physiological weakness in triploid oysters in winter. Interestingly, the mortality period now begins around 16 °C rather than19 °C, as it did before 2008, simultaneous to a huge increase of triploid oysters in growing areas observed in these same recent years. Physiology of triploids in relation to their production technology and environmental conditions should therefore be investigated in depth.

These different hypotheses, which are partly based on technical reports as well as published papers, should be further documented.

However, in such extreme pathogenic conditions, diploid (2n) R oysters continue to resist more than 2n natural (unselected) oysters, even though the mortality rate is higher than usual for both sets. This means that the fundamental underlying mechanism of resistance is still acting under these new conditions. This once more indicates the potential to select diploid R oysters to counterbalance the pathogen increase. Selection could aim to enhance resistance based either on the criteria proposed in this presentation (strategies 1 and 2), or on other more classic criteria like defence mechanisms.

Numerous defence genes could also provide putative markers for selection (Gueguen et al. 2003; Gonzales 2007; Gueguen et al. 2009; Mateo et al. 2010; Duperthuy et al. 2011; Renault et al. 2011). However, considering the proposed conceptual model, if their expression is limited by ROS production and related energy deficiency, it is probably better to prioritise the selection of genes preventing ROS production (strategy 1), or ROS detoxification genes (strategy 2) because these

approaches will also control the expression of defence mechanisms.

5 Other molluscan pathologies

Although pathologies can be relatively well controlled in terrestrial cultures or in enclosed rearing areas, they are difficult to fight in open marine sites. Most studies are focused on selection against identified pathogens, and necessitate long term research on many different host-pathogen interactions. In contrast, in *C. gigas* summer mortality studies, we learned that the multifactorial process provides possibilities of fighting this pathology by preventing one among a number of different parameters involved in contributing to this mortality risk (temperature, food conditions, stress, genetics, etc. (Samain et al. 2008). This approach would allow us to prevent infections from different pathogens as virus or bacteria.

An important issue arises concerning the possible role of such a multifactorial interaction in other molluscan pathologies. If the r and K concept can be applied to oysters, as such a mechanism is highly conserved though evolution, it could be interesting to document it for other molluscan species. Moreover, the putative role of a similar mechanism should be considered in other cases of pathogenic infection of aquaculture species. The European (flat) oyster (*Ostrea edulis*), a bivalve cultivated directly on the sediment (a potential stress source), is infected by the parasite *Bonamia ostreae* at summer temperatures during its first reproductive period. Recently, da Silva et al. (2009) found differential *Bonamia ostreae* infection levels in *Ostrea edulis* oysters. Spat from broodstock originating from different geographical areas was produced experimentally and reared to adult stages in a common environment. Differences between origins were observed in reproductive strategy. As a whole, this study showed that the two highest infected groups of oysters had the highest percentages of ripe and partially spawning gonads. These conditions look very similar to the *C. gigas* interaction model, and more research should be dedicated to this species to select resistant flat oysters based on the same strategy as that used for *C. gigas* R oysters (selected for resistance to summer mortality) in Morest program, based on reproductive strategy and the ROS detoxification concept as described above. Mass mortalities of cultured zhikong scallops (*Chlamys farreri*) have occurred each summer in most culture areas of northern China since 1996. According to Xiao et al. (2005), "it is possible that the mortalities are caused by a combination of several factors such as stress associated with reproduction, high temperature, overcrowding and poor circulation in the grow out cages, opportunistic invaders or pathogens, and possibly inbreeding". Moreover, the abalone (gastropod) *Haliotis tuberculata* could be another example, with mortality attributed to a pathogenic *Vibrio harveyi* recorded with abalone reproduction and characterised by high seawater temperatures, whereas basal reactive oxygen species production increased significantly (Travers et al. 2008). Summer mass mortality was also reported for the ascidian *Halocynthia roretzi* in culturing grounds of southern coast of Korea during the long period of summer drought and the unusual high water temperature in 1988 (Na et al. 1991).

6 Environmental quality and marine pathologies

Considering marine pathologies more generally, an increase in anthropogenic environmental stressors appears to be associated with an increase in pathological problems in mariculture (8th International conference on shellfish restoration, Brest, ICSR 2005). Long term statistics on river nitrate levels show large increases starting in the 1970s, coinciding with marine pathology problems in developed countries at this time, and the same pattern is repeated around the 1990s in developing countries. Considering that high nutrition and stress during the reproductive stage are critical factors for the health of marine organisms, the combat against pathologies should first be oriented against environment-related trophic changes, and other associated anthropic stresses.

7 Conclusion

This approach has provided a better understanding of physiology-environment-pathology interactions, a better definition of research priorities and direction to improve knowledge, and the applied means to fight pathologies in marine aquaculture. The conceptual model based on energy balance control and "soma-germ cell conflict" could explain the numerous interacting factors described in the *C. gigas* summer mortality model (Morest program). These numerous factors offer different ways of countering *C. gigas* summer mortality and the different pathogens involved by focusing on priorities for environmental restoration and protection to reduce stressors and excess nutrient inputs, simultaneously adapting husbandry practices to disrupt the interaction model that would otherwise lead to mortality risks, and perform selective breeding based on reproductive strategy and/or ROS detoxification processes. New complementary criteria for selection are proposed for investigation (on energy balance, reproduction, mitochondria and oxidative stress resistance). Such strategies could form a useful complement to classic selection aiming to improve resistance to identified pathogens or to select according to immune capacities. According to the literature, the mechanisms in question appear to have been highly conserved over evolution. This approach would therefore offer possibilities for other mollusc species and probably other aquaculture species, such as shrimps and fishes, facing opportunistic pathogens.

Acknowledgements. I would like to thank Réjean Tremblay and the scientific committee of Physiomar 2010 for their kind invitation to open this meeting in Quebec. I thank all the colleagues that have contributed to the Morest project and the new generation now developing molecular approaches in the framework of this subject. I congratulate them for the very interesting new results we shared during this meeting. I thank Helen McCombie for reviewing the English.

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