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Characterisation of physiological and immunological differences between Pacific oysters (*Crassostrea gigas*) genetically selected for high or low survival to summer mortalities and fed different rations under controlled conditions

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Abstract

Within the framework of a national scientific program named "MORtalités ESTivales de l'huître creuse *Crassostrea gigas*" (MOREST), a family-based experiment was developed to study the genetic basis of resistance to summer mortality in the Pacific oyster, *Crassostrea gigas*. As part of the MOREST project, the second generation of three resistant families and two susceptible families were chosen and pooled into two respective groups: "R" and "S". These two groups of oysters were conditioned for 6 months on two food levels (4% and 12% of oyster soft-tissue dry weight in algal dry weight per day) with a temperature gradient that mimicked the Marennes–Oléron natural cycle during the oyster reproductive period. Oyster mortality remained low for the first two months, but then rapidly increased in July when seawater temperature reached 19 °C and above. Mortality was higher in "S" oysters than in "R" oysters, and also higher in oysters fed the 12% diet than those fed 4%, resulting in a decreasing, relative order in cumulative mortality as follows; 12% "S" > 12% "R" > 4% "S" > 4% "R". Although the observed mortality rates were lower than those previously observed in the field, the mortality differential between "R" and "S" oysters was similar. Gonadal development, estimated by tissue lipid content, followed a relative order yielding a direct, positive relationship between reproductive effort and mortality as we reported precedently by quantitative histology. Regarding hemocyte parameters, one of the most striking observations was that reactive oxygen species (ROS) production was significantly higher in "S" oysters than in "R" oysters in May and June, regardless of food level. The absence of known environmental stress under these experimental conditions suggests that the ROS increase in "S" oyster could be related to their higher reproductive activity. Finally, a higher increase in hyalinocyte counts was observed for"S" oysters, compared to "R" oysters, in July, just before mortality. Taken together, our results suggest an association of genetically based resistance to summer mortality, reproductive strategy and hemocyte parameters.

Keywords: *Crassostrea gigas*; Genetic selection; Hemocyte parameters; Reactive oxygen species (ROS); Reproduction; Summer mortality

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1. Introduction

Summer mortalities of the Pacific oyster, *Crassostrea gigas*, were first reported in the 1940s in Japan (Koganezawa, 1974), in the late 1950s on west coast of North America (Glude, 1974; Koganezawa, 1974; Cheney et al., 2000), and in early 1980s in France (Goulletquer et al., 1998). These seasonal mortalities affect both adults and juveniles, with no specific clinical signs of disease.

To date, some pathogenic agents have been detected and isolated during summer mortality events (Elston et al., 1987; Friedman and Hedrick, 1991; Lacoste et al., 2001; Le Roux et al., 2002; Waechter et al., 2002; Gay et al., 2004; Garnier et al., 2007), but these organisms have not been clearly and systematically implicated in mortalities. One common feature of these summer mortality events is that they are associated with at least one of the following parameters: high trophic conditions, elevated summer temperatures, and coincidence with the period of sexual ripeness in oysters (Soletchnik et al., 1999, 2003, 2005). Only a few experimental studies, however, have confirmed this contention (Lipovsky and Chew, 1972; Perdue et al., 1981). The high energetic cost associated with reproduction, combined with high summer temperatures, was hypothesized to weaken the oysters and make them more susceptible to opportunistic pathogens (Perdue et al., 1981; Koganezawa, 1974). Findings from MOREST, a national multidisciplinary program initiated in France in 2001, show that other environmental and potentially-stressful factors associated with rain, aquaculture practices, and sediment quality also seemed to be related to oyster summer mortality (Soletchnik et al., 2003, 2005).

Moreover, summer mortality was found to be linked, to some extent, to genetic variability in oysters (Beattie et al., 1980; Hershberger et al., 1984; Ernande et al., 2004). During the MOREST project, bi-parental families were bred in the hatchery following a half-sib nested design and deployed in three rearing sites (Ronce, Rivière d'Auray and Baie des Veys) during the summer of 2001. At the end of the summer period, family had the largest variance-component for survival (46%) (Dégremont et al., 2005). Heritability of spat survival was estimated to be very high (Dégremont et al., 2007). In 2002, families selected for high (called "R" for resistant) or low ("S" for susceptible) survival were used to produce a second generation which was tested in the field under similar conditions as the previous year. In October, the mortality of the "R" oysters was 2%, 12% and 6% in Ronce, Rivière d'Auray, and Baie des Veys sites, respectively, but consistently higher, 23%, 42% and 32% for the "S" oysters. Once again, second generation family represented the largest variance (61%), and this second field experiment confirmed that survival is a highly heritable trait (Dégremont, 2003). Other family-based, selective-breeding programs also have shown high broad-sense heritability for survival in *C. gigas* (Evans and Langdon, 2006) and *C. virginica* (Dégremont, personal communication) and realized heritability for yield, a parameter combining survival and growth, in *C. gigas* on the US West Coast (Langdon et al., 2003). Clearly a significant genetic effect was observed in the complex summer mortality phenomenon.

Little information is available, however, on the physiological basis of divergent selection for "S" vs "R" oysters. Within the framework of MOREST, several field and laboratory studies were performed to compare various biological parameters in "R" and "S" oyster families, or groups of families, to explain survival differences (Samain et al., 2007). As mentioned before, the high energetic cost associated with reproduction, combined with high summer temperatures and other stresses, is suspected to weaken the oysters and make them more susceptible to opportunistic pathogens. As the capability of an oyster to react to diseases, injuries or parasite infestation depends upon innate, humoral and cellular defence mechanisms (Cheng, 2000; Chu, 2000), it appears pertinent to assess whether or not survival traits include better immune responses.

One approach to assessing immune responses of oysters is to measure hemocyte parameters (descriptive and functional). Indeed, hemocytes are considered to be the main cellular mediators of the defence system in bivalves (Volety and Chu, 1995; Cheng, 1996), responsible for recognition, phagocytosis, and elimination of non-self particles by microbicidal activities (Pipe, 1992; Cheng, 2000; Chu, 2000). Recently, we reported that some hemocyte activities (phagocytosis, adhesion) decreased during gametogenesis, especially when gonads approach ripeness (Delaporte et al., 2006a; Gagnaire et al., 2006). Other studies (Enriquez-Diaz, 2004) demonstrated by histological analysis that "S" families from the first generation exhibited earlier and higher gonad development than "R" families when reared together in Rivière d'Auray (France).

In the present study, the objective was to assess whether or not different survival of summer mortalities is related to reproductive, energetic, or immune status evaluated by quantifying biochemical and hemocyte parameters. These parameters were investigated on a subsample of animals from a group of three "R" families and a group of two "S" families produced by divergent selection in the field. These groups were compared in experimental conditions during the period of active