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Short communication

Size and genotype affect resistance to mortality caused by OsHV-1 in *Crassostrea gigas*

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ABSTRACT

As with summer mortalities reported in France between 2001 and 2006, mortality caused by the Ostreid herpesvirus 1 (OsHV-1) in *Crassostrea gigas* affects mostly juveniles, although adults can also be impacted to a small extent. This could suggest that both mortalities have similar causes and that establishment of resistance, in particular to the Ostreid herpesvirus 1 (OsHV-1), depends on either the size or the age of oysters. The present study reports an investigation of both size and age using three cohorts produced during winter and three produced during summer. Each cohort contained oysters genetically selected to be resistant or susceptible to the summer mortality phenomenon, as well as unselected control oysters. Any abnormal mortality was recorded between production and placement in the field. Transfer to field conditions was then made over thirty deployments between July 2009 and September 2011. All mortalities occurred when seawater temperature was above 16 °C, which was termed the 'risk' period. For all deployments made during the risk period, mortality was observed within two weeks post-deployment and most episodes lasted over a week. For deployments made outside of the risk period, mortality occurred as soon as the next risk period began. The absence of detection of OsHV-1 at deployment, the presence of a high viral load of OsHV-1 ($>10^{+6}$ DNA copies per mg of fresh tissue) on moribund oysters sampled during peak mortality, and the mortality kinetic all suggest that the mortalities can be attributed to this pathogen alone. The major finding of this study was that the resistance to mortality caused by OsHV-1 increased with both age and size, suggesting a maturation of the immune system against the virus. In field conditions, the relationship between mortality and size was stronger than the relationship between mortality and age. Regression equations of oyster size or age at the onset of the mortality event were derived to estimate the mortality due to OsHV-1. Although larger animals always tended to be more resistant to OsHV-1 than smaller ones, mortality in unselected oysters remained high (>70%) for the size range 0–10 g. Selective breeding to improve resistance to OsHV-1 remains the best way to significantly reduce mortality; however, prudent management strategies for oyster growers could also potentially offer viable solutions. For example, deploying juveniles at a site favouring the growth of oysters after the threat of exposure to OsHV-1 has passed (i.e. at the end of the risk period), and by using cultural practices favouring high growth and/or a site for which the risk period is short due to the seawater temperature. Use of triploid oysters or lines selected for higher growth is also discussed.

1. Introduction

Summer mortality in *Crassostrea gigas* has been reported throughout the world, in Japan in 1915 (Koganezawa, 1975; Takeuchi et al., 1960), the USA in the late 1950s (Glude, 1975), France during the 1980s (Maurer et al., 1986) and more recently in Germany (Daehne et al., 2009), Ireland (Malham et al., 2009) and Wales (Cotter et al., 2010). The role of pathogens was not clear until the investigation of the more severe mortality outbreaks occurring in France since 2008, in which the Ostreid herpesvirus 1 (OsHV-1) was frequently detected (Segarra et al., 2010). Similar mortalities also occurred in other countries of Europe, in Australia and New Zealand, and on the west coasts of the USA at this time (Burge and Friedman, 2012; Lynch et al., 2012; Martenot et al., 2012; Peeler et al., 2012; Roque et al., 2012; Cameron and Crane, 2011).

Breeding investigations revealed high genetic basis for survival during summer mortality events in juvenile *C. gigas* and a positive response to selection to improve or decrease survival (Dégremont et al., 2007, 2010b). Oyster line bred from families selected in 2001 to be either resistant or susceptible to summer mortality, hereafter referred to as R and S, respectively, still exhibit contrasted survival in the context of the more severe mortality outbreaks occurring in France since 2008 (Dégremont, 2011). It was also shown that R oysters were capable of limiting the infection by OsHV-1 in comparison to S and unselected oysters, indicating their resistance to the disease and supporting the

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hypothesis that summer mortality and the particularly severe mortality outbreaks observed in juvenile *C. gigas* since 2008 in France probably result from the same phenomenon (Dégremont, 2011).

Indeed, the first disease investigation clearly showed relationship between OsHV-1 detection and *C. gigas* spat mortality in France between 1998 and 2006 (Garcia et al., 2011). The detection of OsHV-1 in moribund oysters during mortality events in laboratory trials and in nursery was also reported in 1995 (Renault et al., 1995) and from 2001 to 2003, as strong correlations between laboratory and field mortality (Dégremont, 2003; Dégremont et al., 2010a). OsHV-1 was also detected in three full-sib families of the R and S lines in 2006, and QTL for resistance to summer mortality and OsHV-1 load were then found using these lines (Sauvage et al., 2009, 2010). Additionally, the kinetics of the mortality in laboratory trials in 2002 and 2003 were similar to those found in the field in 2009, with 90% of the cumulative mortality occurring within a week (Dégremont, 2011; Dégremont et al., 2010a).

Disease investigation showed higher susceptibility to OsHV-1 in juveniles than adults (Renault and Novoa, 2004), and genetic investigation showed similar results with juvenile oysters being more susceptible to summer mortality than adults (Dégremont et al., 2010c). This last result revealed the importance of the age and/or size for the establishment of the resistance during the oyster development; however, several questions remain unanswered. For example, at what age do R and S oysters show contrasted survival? Is the difference present from hatching or does appear at a later stage of development? How does phenotypic variance evolve through the development of *C. gigas*? Which is the key factor for OsHV-1-related mortality: age or size? Using the lines selected for contrasted survival/resistance and unselected controls, this study aimed to address these questions through an investigation of size and age at the juvenile stage in field conditions.

2. Materials and methods

2.1. Biological materials

To distinguish the relative influence of age and/or size on mortality, several cohorts were produced either during winter or summer and deployed several times in order to provide a large range of ages and sizes as possible, and also to expose oysters from the same genetic groups with similar sizes but different ages to the same OsHV-1-related mortality risks.

From 2009 to 2011, one cohort per year was produced during the winter, each consisting of three groups (selected R, selected S, and unselected, hereafter referred to as 'C') within which there were two replicate batches each. Additionally, three cohorts were produced during the summer, one in 2009 and two in 2010, and the number of batches per group is indicated in Table 1. Additional batches were also produced from the summer spawnings, but were not allocated to the above groups as they were issued from crosses between C and R oysters and were either diploid or triploid. These additional oysters were grown alongside the experimental oysters to observe the trend of mean mortality according to their age and/or size. Unselected oysters were sampled from a wild population in Marennes-Oléron Bay in 2009 and 2010. For each cross, an average of 36 parents was used. Larval rearing and settlement stages took place at the Ifremer hatchery in La Tremblade. The spat were then transferred and maintained at the Ifremer nursery in Bouin until field deployment. Any abnormal mortality was recorded from the spawn to the field deployment. Further details on these steps and on selection criterion are given in Dégremont et al. (2005, 2007, 2010a, 2010b). The R oysters of cohorts 3 and 4 were produced using survivors of the R batches tested in cohort 1; likewise, the R oysters of cohorts 5 and 6 were produced using the survivors of cohort 3. This meant that the later cohorts had one or two more rounds of mass selection for survival in comparison with the R batches of cohorts 1 to 2.

Table 1
Key dates and mortality (%) of the six cohorts.

Cohort	Date of spawning	Date of field deployment	Date of mortality[a]	Nb of batches tested[b]	Mean mortality (%)			
					All (±SD)[b]	R[b]	C[b]	S[b]
1	02/09	07/09	WTW	6/2/2/2	75 ± 20	51	80	91
		08/09	WTW[c]	6/2/2/2	58 ± 39	21	60	95
		09/09[c]	WTW[c]	6/2/2/2	51 ± 44	5	53	94
		10/09[c]	06/10[c]	6/2/2/2	23 ± 13	8	33	29
2	08/09	03/10[c]	06/10	4/0/1/1	83 ± 12	NA	93	92
		04/10[c]	06/10	4/0/1/1	81 ± 10	NA	86	93
		05/10[c]	06/10[c]	4/0/1/1	85 ± 9	NA	91	94
		06/10[c]	WTW[c]	4/0/1/1	86 ± 14	NA	96	96
		07/10[c]	WTW	4/0/1/1	74 ± 18	NA	80	97
		08/10[c]	WTW	4/0/1/1	55 ± 18	NA	71	68
		09/10[c]	WTW	4/0/1/1	32 ± 16	NA	45	44
3	03/10	06/10[c]	WTW[c]	6/2/2/2	80 ± 23	54	92	95
		07/10[c]	WTW	6/2/2/2	80 ± 27	49	95	97
		08/10[c]	WTW	6/2/2/2	73 ± 32	36	88	95
		09/10[c]	WTW	6/2/2/2	63 ± 40	17	77	94
		10/10[c]	05/11	6/2/2/2	30 ± 21	7	41	43
4	07/10	03/11	05/11	6/1/1/1	72 ± 31	19	97	95
		04/11	05/11	6/1/1/1	59 ± 26	17	86	80
		05/11	05/11	6/1/1/1	61 ± 30	10	87	95
5	08/10	03/11	05/11	4/1/1/0	67 ± 30	26	94	NA
		04/11	05/11	4/1/1/0	70 ± 27	31	93	NA
		05/11	05/11[c]	4/1/1/0	54 ± 36	6	88	NA
		06/11	WTW	4/1/1/0	51 ± 40	9	91	NA
		07/11	WTW	4/1/1/0	58 ± 27	25	81	NA
		08/11	WTW	4/1/1/0	43 ± 33	7	81	NA
		09/11	WTW + 05/12	4/1/1/0	25 ± 15	12	43	NA
6	02/11	06/11	WTW	6/2/2/2	63 ± 45	16	80	93
		07/11	WTW	6/2/2/2	64 ± 38	22	83	88
		08/11	WTW	6/2/2/2	62 ± 37	15	85	87
		09/11	WTW	6/2/2/2	54 ± 42	7	78	78

NA: not available.
[a] WTW: within two weeks post-deployment.
[b] All batches (R, C, S and batches derivate from progeny from a cross made between R and C), and R, C and S batches, respectively.
[c] OsHV-1 disease diagnoses on live oysters at deployment or on moribund oysters during mortality outbreaks.

2.2. Field study

All oysters were all tested at Agnas in Marennes-Oléron Bay (45°52′23″N, 1°10′15″W) using bags attached to racks. For each cohort, several deployments from nursery to field were made at monthly intervals, as detailed in Table 1. For example, oysters of cohort 1 were deployed in the field from July to October 2009, while those of cohort 2 were deployed from March to September 2010. At each deployment, the progenies were represented by two bags of 150 oysters each and the total weight of the oysters was recorded. Oysters were checked two weeks post-deployment, and mortality and total weight of the live oysters were recorded two weeks post-mortality for the spring deployments, and one month after the mortality peak. The seawater temperature was recorded throughout the study using a YSI probe #6600.

2.3. OsHV-1 detection and quantification

Live oysters were sampled for some batches of cohorts 1, 2 and 3 at the IFREMER nursery before deployment in the field, representing a total of 408 oysters (Table 1). Moribund oysters were also sampled during the period of peak mortality each year (n = 72 oysters, Table 1). Oysters were individually diagnosed for OsHV-1 using the real-time PCR technique developed by Pépin et al. (2008) and the protocol described in Sauvage et al. (2009).