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Comparative histological study of gametogenesis in diploid and triploid Pacific oysters (*Crassostrea gigas*) reared in an estuarine farming site in France during the 2003 heatwave

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ABSTRACT

We compared the temporal dynamics of gametogenesis in diploid and triploid Pacific oysters (*Crassostrea gigas*) using histology. Oysters were reared in an estuarine farming site in Brittany over 3 years and their gametogenesis was monitored over the exceptionally hot summer of 2003. Both diploids and triploids showed active gametogenesis, but a high proportion of triploids remained at early stages of gonad development. Gametogenesis of triploids was characterized by the simultaneous occurrence of gonias and immature cytes together with mature gametes rather than overall retardation, though some triploids showed complete gonad maturation. Evidence of spawning was seen in both groups, and the quantity of germinal products emitted appeared to be similar in triploids and diploids, though triploids were much heavier than diploids. Our study supports previously reported experimental observations and suggests that gonad development in triploid Pacific oysters can be enhanced when these oysters are reared in the field under unusually warm conditions.

1. Introduction

Genetic improvement of *Crassostrea gigas* through triploidy leads to faster growth (Nell and Perkins, 2005; Garnier-Géré et al., 2002) and reduced gametogenesis (Allen and Downing, 1986, 1990; Goulletquer et al., 1996). Faster growth rates have been reported for both chemically induced triploid oysters and those produced by diploid × tetraploid crosses. Triploid oysters appear to be at a particular advantage in sites with high trophic richness (Eudeline, 2004). It is very likely that the reduction in gametogenesis contributes directly to the faster growth of triploids compared to diploids, allowing a reorientation of the energetic allocation from reproduction to growth (Allen and Downing, 1986; Hawkins et al., 2000; Honkoop, 2003). The reduction of gametogenesis during the reproductive period also improves marketability of these oysters during the reproductive period (i.e. summer) and limits the propagation of these individuals in the natural environment. It should, however, be noted that triploidy is not considered as an efficient method of genetic confinement in oysters (Anderson et al., 2004) since it only leads to partial sterility and because triploids can revert to a diploid stage (Allen et al., 1996, 1999).

Triploid oysters are only partially sterile and retarded gametogenesis of such oysters has been observed in both Humbolt Bay (USA) (Allen and Downing, 1990) and in Tasmania (Gardner et al., 1994). Guo and Allen (1994) estimated that relative fecundity of triploid females was around 2% of the level in their diploid control, with an average of 2.261 million stripped eggs per female in 2-year-old triploid oysters. Despite the lower reproductive potential of triploids relative to diploids, these authors noticed a high variation in reproductive effort between triploid females. While some triploid females had no mature oocytes, others had up to 21 million oocytes that was equivalent to the mean fecundity of the diploid female controls.

In diploid Pacific oysters, gametogenesis is under control of both exogenous signals from environment and endogenous physiological factors. Temperature and photoperiod drive the internal clock of Pacific oysters, while body condition also influences reproductive development via feedback from other metabolic compartments (Pouvreau et al., 2006; Fabioux et al., 2005; Enriquez-Diaz, 2004; Ren and Ross, 2001). Temperature has long been known as a major factor controlling gametogenesis (Mann, 1979; Chávez-Villalba et al., 2002a,b; Fabioux et al., 2005) and elevation of water temperature stimulates oocyte growth (Chávez-Villalba et al., 2002b). The effect of environmental factors known to control gametogenesis in diploid oysters should be considered in any

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study of gametogenesis in triploid oysters because effective gametogenesis can occur in triploid Pacific oysters and that individuals exhibit a high variation in reproduction effort.

Following the recent paper by Duchemin et al. (2007), focussing on seasonal variations of immune parameters in diploid and triploid Pacific oysters, the present study further documents the temporal dynamics of gametogenesis in triploid oysters raised in an estuarine rearing site (Aber Belon, South Brittany, France). Our study was performed during the particularly hot and dry summer of 2003, which potentially favoured gametogenesis in triploid oysters.

2. Materials and methods

2.1. Biological material and sampling

Diploid oysters were collected from natural settlement and triploid oysters were purchased from a commercial hatchery thus representing the usual sources of these types of seed cultivated by oyster farmers in France. The triploid oysters had been produced by chemical induction using cytochalasin-B (Allen et al., 1989). Both groups of oysters were 3 years old at the beginning of the study (May 2003) and presented a very similar initial mean dry meat weight (DMW) (1.46±0.44 and 1.56± 0.59 g for diploids and triploids respectively). Oysters were cultured in Aber Belon (South Brittany, France) following local practices, off-bottom, using iron frames ('tables') onto which the oyster mesh bags are attached (Fig. 1). More than fifty individuals from each of the groups were randomly sampled from a very large number of commercially farmed oysters twice a month during the intensive gonad maturation period (i.e. from May to July), then monthly from August 2003 to April 2004.

2.2. Environmental monitoring

Environmental monitoring was performed during the sampling period by a temperature sensor localized in Riec-sur-Belon close to the farming site where the experimental oysters were reared. The relevant dataset was extracted from the Quadrige database developed by Ifremer (http://www.ifremer.fr/delao/francais/valorisation/quadrige/index.htm) and reflected the exceptional heat experienced in the study area in summer 2003 and its effect on seawater temperature.

2.3. Biometrical measures

Dry meat weight and shell weight of 30 individuals randomly sampled out of the 60 oysters from each group (diploid and triploid) were measured to estimate their Walne and Mann condition index (C.I.):

$$\text{C.I.} = (\text{dry meat weight} \times 1000)/\text{shell weight}.$$

Individual shell weight was checked to insure that no significant difference in flesh or shell growth could reveal a sampling bias.

2.4. Ploidy analysis

Chemical induction of triploidy rarely produces 100% triploid batches (Allen et al., 1989). In order to determine the ploidy of individual oysters sampled in the triploid group and exclude diploid individuals from further histological analyses, up to thirty oysters from this group were individually tested by flow cytometry using the propidium iodide method, as described by Utting and Child (1994), until twenty triploid oysters were obtained for histology.

2.5. Histological analysis and cell diameter measurement

At each sampling point, 20 individuals per group were sampled following the ploidy analysis for histological analysis. A piece of the visceral mass was placed in Bouin's fixative for 48 h before routine histological processing. Paraffin embedded pieces of tissue were cut into 5 μm sections and then coloured with Masson trichrome.

C. gigas is normally a protandrous alternate hermaphrodite. Microscope observation allowed us to classify individuals as males, females or undetermined. Reproductive stage was determined following a qualitative classification (5 stages: 0 to 4) adapted from Mann (1979) and Lango Reynoso et al., (2000) (Table 1). These guidelines were based on those originally described for *Crassostrea virginica* (Kennedy and Battle, 1964 in Mann, 1979).

In order to describe female reproductive stages more precisely, the diameter of germinal cells (Lango Reynoso et al., 2000) was measured for 60 cells per female on 10 randomly sampled females of each group in June 2003. Histological slides were observed under a microscope at 100× and pictures were recorded with a digital camera (Sony). The pictures were then processed on a Silicon Graphics station using Visilog

Fig. 1. Localization of the sampling site.