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Variability of haemocyte and haemolymph parameters in European flat oyster *Ostrea edulis* families obtained from brood stocks of different geographical origins and relation with infection by the protozoan *Bonamia ostreae*

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Abstract A research project to compare productive traits (growth and mortality), disease susceptibility and immune capability between *Ostrea edulis* stocks was performed. This article reports the results on the immune capability and its relation with infection by the intrahaemocytic protozoan *Bonamia ostreae*. Four to five oyster spat families were produced from each of four European flat oyster populations (one from Ireland, one from Greece and two from Galicia, Spain) in a hatchery. The spat were transferred to a raft in the Ría de Arousa (Galicia) for on growing for 2 years. Total haemocyte count (THC) and differential haemocyte count (DHC) were estimated monthly through the second year of growing-out. Three types of haemocytes were distinguished: granulocytes (GH), large hyalinocytes (LHH) and small hyalinocytes (SHH). Significant correlations between the mean relative abundance of GH and SHH of the families and the mean prevalence of *B. ostreae*, the overall incidence of pathological conditions and the cumulative mortality of the families were found; these correlations supported the hypothesis that high %GH and low %SHH would enhance oyster immune ability and, consequently, would contribute to lower susceptibility to disease and longer lifespan. Infection by *B. ostreae* involved a significant increase of circulating haemocytes, which affected more markedly the LHH type. The higher the infection intensity the higher the %LHH. This illustrates the ability of *B. ostreae* to modulate the immune responses of the *O. edulis* to favour its own multiplication. A significant reduction of the phenoloxidase activity in the haemolymph of oysters *O. edulis* infected by *B. ostreae* was observed. Nineteen enzymatic activities in the haemolymph of *O. edulis* and *Crassostrea gigas* (used as a *B. ostreae* resistant reference) were

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measured using the kit api ZYM®, Biomerieux. Qualitative and quantitative differences in enzyme activities in both haemocyte and plasma fractions between *B. ostreae* noninfected *O. edulis* from different origins were recorded. However, no clear positive association between enzyme activity and susceptibility to bonamiosis was found. The only enzyme detected in the resistant species *C. gigas* that was not found in the susceptible one *O. edulis* was β-glucosidase (in plasma). *B. ostreae* infected *O. edulis* showed significant increase of some enzyme activities and the occurrence of enzymes that were not detected in noninfected oysters. These changes could be due to infection-induced enzyme synthesis by the host or to enzyme synthesis by the parasite.

Introduction

The disease caused by the protozoan parasite *Bonamia ostreae* contributed to the exhaustion of most European flat oyster *Ostrea edulis* populations in Galicia and is the main constraint for oyster farming in that region. Unsuccessful strategies to fight against bonamiosis were implemented in France [1] and Holland [2,3], including zootechnical prophylaxis and eradication attempts. However, two selective breeding programmes for bonamiosis resistance, one in France [4—7] and another in Ireland [8], gave rise to encouraging results for the oyster industry, and highlighted the possibility to grow flat oysters in areas affected by bonamiosis, with higher survival and lower *B. ostreae* prevalence and infection intensity [9]. Accordingly, the Centro de Investigacións Mariñas decided to develop a selective breeding programme to produce oysters *O. edulis* with increased tolerance to bonamiosis [10]. As a previous stage to this programme, evaluation of the variability of productive traits, disease susceptibility, and immune capability through oyster populations was performed, because particular populations could be favourable for the programme. Four geographic origins were chosen for variability assessment, including extreme and intermediate locations in the European flat oyster geographic range: Greece (Eastern Mediterranean), Ireland (Northern Atlantic), and two areas in Galicia, Spain. Oysters from those locations were used as brood stocks and 19 full- or half-sib families were produced (4—5 families from each origin). The evaluation of productive traits and disease susceptibility showed significant differences in growth, mortality and susceptibility to bonamiosis and other diseases, both between origins and between families within origins; these results have been published elsewhere [10].

Evaluation of the immune capability was also considered in the experimental design because it could explain the differences in disease susceptibility that could be found. The immune system of bivalve molluscs relies upon haemocytes, which play an important role against parasites by phagocytosis or encapsulation, with subsequent destruction via hydrolytic enzymes [11], reactive oxygen intermediates (ROIs) [12] and antimicrobial peptides [13]; in addition, humoral factors consisting of non-self recognition molecules and immune effectors, such as lectins, opsonins, and components of the prophenoloxidase (proPO) system may play a key role in immune reactions [14]. Two main haemocyte types can be distinguished in the haemolymph of bivalves: granular haemocyte (GH) and hyaline haemocyte (HH) [15], which also applies to *O. edulis* [16].

This paper reports the inter (between origins) and intra (between families within origins) population variability in a number of immune parameters: total haemocyte count (THC), differential haemocyte count (DHC), 19 hydrolytic enzyme activities in the cell and plasma fractions of the haemolymph, and phenoloxidase activity. The association of these parameters with the *B. ostreae* infection was also analysed.

Materials and methods

Production of oyster families

Four oyster populations were selected as brood stock for the experiments: one in the North of Ireland (IR), one in Greece (GR) and two in Galicia (NW Spain), one from the Ría de Ortigueira (OR) and another from Coroso (CO) (Ría de Arousa). The oysters from each origin were brought to the hatchery facilities of the Centro de Investigacións Mariñas in December 2000 and handled as described by da Silva et al. [10] to produce various families of spat from each origin. Briefly, the oysters were distributed into five trays per origin with 15—20 individuals per tray and conditioned for spawning. Batches of recently spawned larvae originated from a single mother were collected from each tray. Thus, all the larvae within each batch were half or full sibs. Every larval family was separately reared. Once spat surpassed 1 cm in height they were transferred to a raft for on growing. A total of 19 families were produced, 5 from each origin, except for IR, for which only 4 families were obtained.

Oyster on growing

In September 2001, approximately 4000 individuals from each family were transferred to a raft located near Cambados (Ría de Arousa, Galicia, NW Spain), in an oyster culture area heavily affected by bonamiosis since the 1980s [17]. Oysters were cultivated up to September 2003, being handled as described by da Silva et al. [10] through the on growing process. Samples of oysters were randomly taken (monthly up to June 2002 and quarterly since then) to estimate growth by measuring their height and whole weight. Estimation of mortality was accomplished monthly up to June 2002, quarterly since then, by counting dead and live individuals. A number of oysters (five oysters up to July 2002 and six since then) from each family were randomly taken monthly for disease diagnosis. Sampling for measuring