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Effects of temperature and salinity on haemocyte activities of the Pacific oyster, *Crassostrea gigas* (Thunberg)

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Abstract

The Pacific oyster, *Crassostrea gigas*, is extensively cultivated and represents an important economic activity. Oysters are reared in estuarine areas, subjected to various biotic and abiotic factors. One of the limiting factors in aquaculture is mortality outbreaks, which may limit oyster production, and the causes of these outbreaks are not completely understood. In this context, the effects of temperature and salinity on Pacific oyster, *C. gigas*, haemocytes, were studied. Haemocytes are the invertebrate blood cells and thus have been shown to be involved in defence mechanisms. Flow cytometry was used for monitoring several haemocyte parameters. An increase of temperature induced an increase of haemocyte mortality, in both in vitro and in vivo experiments. Temperature modulated aminopeptidase activity. An in vitro decrease of salinity was associated with cell mortality. During the course of in vivo experiments, an increase of phagocytic activity was reported at 15‰ and 50‰. Environmental physical parameters may modulate haemocyte activities.

Keywords: Pacific oyster; *Crassostrea gigas*; Haemocyte; Temperature; Salinity; Flow cytometry; Cellular activity

1. Introduction

Shellfish farming represents an important economic activity around the world. Among shellfish, the Pacific oyster, *Crassostrea gigas,* is the most cultivated species. In France, *C. gigas* was introduced in the 1970s to

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replace the Portuguese oyster, *C. angulata* [1]. France ranks fourth worldwide in the production of *C. gigas* with 150 000 tons produced annually. However, oyster production may be subjected to various limiting factors including mortality outbreaks. For several decades now, French Pacific oyster livestock has presented abnormal mortality outbreaks during the summer period. This phenomenon, called summer mortality, has also been reported in North America and Japan since the 1940s [2–4]. Authors have hypothesised that summer mortality outbreaks are the result of multiple factors, including elevated temperatures, physiological stress associated with sexual maturation, aquaculture practices, pathogens or pollutants [5]. The Pacific oyster, *C. gigas,* is mostly reared in estuaries that are continually contaminated by pollutants [6]. Estuaries are also subjected to important variations of abiotic environmental factors, including temperature and salinity. *C. gigas* is an osmo- and thermo-conformer species [7]. In oysters natural habitat, salinity fluctuates with tidal cycles, rainfall and with drainage from adjacent terrestrial sites [8]. In summer period, temperature can reach high values. Oysters are sessile benthic animals and as such are continually exposed to physico-chemical modifications of the environment. Physical stress such as tidal exposure, which modify temperature and salinity, can affect marine invertebrate defence mechanisms [9].

Bivalve defence mechanisms involve circulating blood cells, the so-called haemocytes [10]. In *C. gigas*, two types of haemocytes can be differentiated on the basis of morphological features: hyalinocytes and granulocytes [10]. Haemocytes constitute one of the main line of defence against non-self particles. They are involved in phagocytosis and encapsulation of foreign material [10–12]. They also contain hydrolytic enzymes and produce reactive oxygen species (ROS), which play a key role in pathogen degradation [13–15]. They have been used as immune capacity indicators in many bivalve species [16–19].

Studies have previously been conducted on the effects of temperature and salinity on bivalve haemocytes [20–23]. Since bivalves are both osmo- and thermo-conformers, haemolymph readily acquires salinity and temperature of the external environment [7]. In fact, haemocytes found in haemolymph and in tissue sinuses are exposed to temperature and salinity variations that occur in the environment. High water temperatures inhibit haemocyte spreading and locomotion in the eastern oyster, *Crassostrea virginica* (Gemlin) [24] while variations of temperature can also affect haemocyte counts and phagocytic activity in *Ostrea edulis* and *Ruditapes philippinarum* [25,26]. On the other hand, elevated salinity increased the time for spreading and reduced haemocytes locomotion towards target particles and may therefore pose an additional stress [27] and may also reduce oyster defence capacities and leave them more susceptible to parasites [27]. Moreover, the susceptibility of *C. virginica* to the protozoan parasites *Perkinsus marinus* and *Haplosporidium nelsoni* is influenced by temperature and salinity [24,28,29].

In this study, the effects of temperature and salinity on the Pacific oyster, *C. gigas*, and haemocyte parameters were investigated. Haemocytes were subjected in vitro to a range of temperatures and salinity. In vivo experiments were also carried out by placing oysters in waters at defined salinities or in incubators at controlled temperatures. Haemocyte parameters were monitored using flow cytometry. This emerging tool has often been used in marine bivalve research to describe haemocyte population characteristics [30,31] or changes associated with pathology or environmental stress [32,33]. Cell mortality, esterase, aminopeptidase activities and phagocytic activity were monitored.

2. Material and methods

2.1. Oysters

Eighteen-month-old Pacific oysters, *Crassostrea gigas*, 7–10 cm in shell length, were produced in the IFREMER hatchery in La Tremblade (Charente-Maritime, France). Temperature experiments were conducted in April and May 2002 and salinity experiments were undertaken in July 2004. For both experiments, oysters were held in tanks receiving a constant flow of external seawater.