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Journal of Experimental Marine Biology and Ecology 339 (2006) 226–235

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.com/locate/jembe

Northward expansion of a marine parasite: Testing the role of temperature adaptation

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Received 18 April 2006; received in revised form 15 July 2006; accepted 2 August 2006

Abstract

The known range of the eastern oyster (*Crassostrea virginica*) parasite, *Perkinsus marinus*, expanded into the northeastern United States in the early 1990s. We used both *in vitro* and *in vivo* data to test the hypothesis that the northward expansion was associated with a low-temperature adapted strain of the parasite. *In vitro* proliferation of nine *P. marinus* isolates from three geographic sites, Massachusetts and New Jersey in the new range, and South Carolina in the historic southern range, was measured at seven temperatures (5 to 35 °C) using a tetrazolium blue dye assay. We wanted to determine if there were between- and within-geographic location differences in the *P. marinus* proliferation rate, and if so, whether they were associated with temperature. We found no evidence of low-temperature adaptation based on the fact that net proliferation rates for isolates from all three geographic locations were similar at temperatures from 5 to 20 °C. On the other hand, at temperatures of 25 to 35 °C, the South Carolina isolates exhibited higher proliferation rates than the northern isolates suggesting possible high-temperature adaptation of parasite strains that are routinely exposed to higher temperatures. Although there was significant within-location variation among isolates, the data tended to group together by geographic location supporting the hypothesis that there is an important regional component to the proliferation rate of *P. marinus* isolates. A survey of published data showed that the temperature at which *in vivo* proliferation was first observed in oysters at sites from the Gulf of Mexico to Massachusetts was typically between 20 and 23 °C with no evidence of a geographic cline. These results lend support to the hypothesis that the recent warming trend in the northeastern US is the most likely explanation for the *P. marinus* range extension.

Keywords: Climate change; *Crassostrea virginica*; *In vitro*; *In vivo*; *Perkinsus marinus*; Range extension; Tetrazolium dye assay

1. Introduction

Range extension of marine organisms occurs for a number of reasons. Probably most organisms have been introduced into new regions by human activities, especially shipping and aquaculture (Cohen and Carlton, 1998; Galil, 2000). Others result from natural factors, including transportation by water currents (Foighil et al., 1999), and some are associated with global change, specifically climate warming (Barry et al., 1995; Stachowicz et al., 2002; Herbert et al., 2003; Zacherl et al., 2003; Diederich et al., 2005). One range extension associated with climate warming is that described for the eastern

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0022-0981/$ - see front matter.
doi:10.1016/j.jembe.2006.08.004

oyster, *Crassostrea virginica* (Gmelin), parasite, *Perkinsus marinus* (Mackin et al., 1950). For most of the time since it was first detected in the late 1940s, *P. marinus* existed from the lower Chesapeake Bay south along the southeastern United States and into the Gulf of Mexico (Ford, 1996; Ray, 1996). In the late 1980s, a northward movement of *P. marinus* was noted in Chesapeake Bay (Burreson and Ragone Calvo, 1996). Between 1990 and 1992, it caused epizootics from Delaware Bay north to Cape Cod; in 1995 it was first reported in Maine (Kleinschuster and Parent, 1995); and in 2002, it was found in Maritime Canada (Stephenson et al., 2003). It is now well established in its new range (Ford and Smolowitz, submitted for publication).

Most described *Perkinsus* species are warm water parasites, being prevalent in the southern United States, the Caribbean, southern Europe, and Australia (DaRos and Canzonier, 1985; Lester et al., 1990; Burreson et al., 1994a; Kim et al., 1998; Littlewood, 2000). Experimental field and laboratory studies of *P. marinus* show a strong temperature dependency for growth, with most rapid proliferation noted at temperatures above about 20 °C (Hewatt and Andrews, 1955; Soniat, 1985; Wilson et al., 1990; Fisher et al., 1992; Chu and La Peyre, 1993). In fact, the failure of *P. marinus* to become established north of the lower Chesapeake Bay before the mid 1980s was thought to be due to intolerance of the low temperatures existing there (Andrews and Hewatt, 1957; Ford and Haskin, 1982). That picture began to change in the mid to late 1980s when *P. marinus* expansion into the northern Chesapeake Bay was associated with a series of droughts and warm winters (Burreson and Ragone Calvo, 1996). The subsequent 1990–92 range extension into the northeastern United States was associated with a period of rapidly warming water temperatures, especially in winter (Cook et al., 1998). Ford (1996) hypothesized that the parasite was introduced historically through repeated shipments of oysters from enzootic southern waters to replenish depleted stocks in the mid-Atlantic and New England. Once there, it remained undetected because low temperature limited its proliferation until the environment became more favorable in the early 1990s. Another hypothesis is that a low-temperature-adapted strain or strains of *P. marinus* had arisen, which permitted epizootics to occur in the relatively cooler waters of the northeastern United States.

The development of *in vitro* propagation methods for *P. marinus* (Gauthier and Vasta, 1993; Kleinschuster and Swink, 1993; La Peyre et al., 1993) has allowed the culture and testing of *P. marinus* isolates from regionally separated areas (Bushek and Allen, 1996b; Reece et al., 2001). In this study, we used *in vitro* cultures from both historical and new ranges, propagated at different temperatures, to test the hypothesis that the northward "expansion" of *P. marinus* was associated with a low-temperature adapted strain of the parasite. Support for this hypothesis would be finding that the new-range isolates proliferated more readily at low temperatures than the historical-range isolates. We also used data from a number of published field studies to estimate the temperature at which *in vivo* proliferation of *P. marinus* begins in oysters from the Gulf of Mexico to Cape Cod, Massachusetts. We reasoned that if a low-temperature-tolerant strain of *P. marinus* were present in the new range, we should observe proliferation, as measured by seasonal prevalence increase, at lower temperatures than those in its historic range.

2. Materials and methods

2.1. P. marinus culture establishment and maintenance

Oysters infected with *P. marinus* were obtained from 3 geographically separated locations: Massachusetts (MA), New Jersey (NJ), and South Carolina (SC) (Fig. 1; Table 1). Although *P. marinus* is found further north and south than these locations, they span much of the temperature range of the parasite and include both the pre-1990 (South Carolina) and the post 1990 (New Jersey and Massachusetts) ranges. At each location, collections were made when the parasite was expected to be proliferating rapidly *in vivo* ($\geq$25 °C). Oysters from Massachusetts and South Carolina were shipped overnight to the Haskin Shellfish Laboratory; those from New Jersey were brought immediately to the laboratory. All oysters were maintained in aerated standing water at 25 °C and at the salinity of the collection site (Table 1). Within a day or two of arrival at the laboratory, the shells of each oyster were notched. Several microliters of hemolymph from the adductor muscle sinuses were examined microscopically for the presence and intensity of *P. marinus*. Oysters with high parasite concentrations were exsanguinated and aliquots of hemolymph placed in sterile 24-well plates with culture medium (Gauthier and Vasta, 1993; Ford et al., 2002) made up to the collection-site salinity (17 to 33 ppt, Table 1). The collection-site salinity was maintained to minimize changes in the parasite's *in vitro* environment that might result in post-collection differential selection among isolates. The isolates were maintained at 25 °C. Wells were monitored and those with *P. marinus* proliferation and without contamination were subcultured into 50-mL T-flasks containing medium made up to the appropriate salinity and also held at 25 °C. Isolates from individual oysters were maintained as separate cultures. Cultures