Journal of Experimental Marine Biology and Ecology 469 (2015) 1–9

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Low temperature tolerance of a sea urchin pathogen: Implications for benthic community dynamics in a warming ocean

Robyn T. Buchwald *, Colette J. Feehan, Robert E. Scheibling, Alastair G.B. Simpson

Department of Biology, Dalhousie University, Halifax, NS B3H4R2, Canada

ARTICLE INFO

Article history:
Received 28 November 2014
Received in revised form 26 March 2015
Accepted 5 April 2015
Available online 22 April 2015

Keywords:
Disease
Host–pathogen interactions
Mass mortality
Paramoeba invadens
Strongylocentrotus droebachiensis
Thermal tolerance

ABSTRACT

Disease outbreaks in the sea urchin *Strongylocentrotus droebachiensis* on the Atlantic coast of Nova Scotia have increased in frequency over the last 35 years, in association with increasing sea temperatures and strong storm events. Previous studies suggest that the causative agent of disease, the pathogenic amoeba *Paramoeba invadens*, cannot withstand winter sea temperatures on this coast, and may be an alien species. Consequently, recurrent epizootics result from repeated introductions of this pathogen, possibly due to advection or vertical mixing associated with storm events. Here we test an alternative hypothesis that *P. invadens* has established resident populations, either through physiological adaptation to low winter temperatures, or because winter minima have increased since 1980. To test this hypothesis, we manipulated culture temperatures of *P. invadens* isolated from moribund sea urchins during a disease outbreak in 2012. Specific growth rates of *P. invadens* in culture at 0.5 to 18.0 °C were similar to those observed in the early 1980s, providing no evidence of physiological adaptation. *P. invadens* declined to extinction in culture after ~1 month at 0.5 °C and 2.0 °C, but survived for 3 months at 3.5 °C, as indicated by recovery of cultures following transfer to optimal temperature. These results indicate a lower temperature tolerance threshold around 2–3 °C, which is consistent with observational data of recurrent mass mortalities of sea urchins in 1983 and 2012 in the absence of a storm but following relatively warm winters. A trend of increasing minimum temperatures in winter indicates that *P. invadens* could undergo a range expansion to establish a resident population in Nova Scotia within 1–2 decades. This is expected to increase the resilience of the kelp-bed state of the benthic ecosystem but result in the complete collapse of the sea urchin fishery.

1. Introduction

Disease outbreaks caused by marine pathogens have increased over the past three decades, with important consequences for the structure and stability of marine ecosystems (Burge et al., 2014). Host–pathogen interactions that result in epizootics can cause shifts in marine community structure (Feehan and Scheibling, 2014; Lafferty et al., 2004; Ward and Lafferty, 2004), although these interactions are poorly understood and may be highly sensitive to environmental change (Burge et al., 2014). Climate change is of urgent concern as physical and chemical changes in the marine milieu (e.g. acidification, warming, increased frequency or severity of storms) are expected to affect the physiology and ecology of hosts and pathogens (Staudinger et al., 2012). It has been hypothesized that increasing sea temperature will lead to an increase in the frequency of disease outbreaks due to pathogen range expansion, increased virulence or decreased host immunity (Burge et al., 2014; Harvell et al., 2002). Given the importance of disease in ecosystem dynamics, understanding the effects of environmental factors, such as temperature, on host–pathogen interactions will better enable us to predict how marine systems will respond to climate change, and to develop appropriate management and response strategies (Burge et al., 2014; Harvell et al., 2009).

Along the Atlantic coast of Nova Scotia, Canada, the green sea urchin *Strongylocentrotus droebachiensis* plays a key role in structuring the rocky subtidal ecosystem (Mann, 1977). At high densities, sea urchins form feeding aggregations that destructively graze kelp beds, causing a phase shift to urchin-dominated barrens (Breen and Mann, 1976; Lauzon-Guay and Scheibling, 2007; Scheibling et al., 1999). This transition was first recorded in St. Margarets Bay (near Halifax) in the early 1970s (Breen and Mann, 1976) and by 1979 barrens spanned the entire Atlantic coast of Nova Scotia (Wharton and Mann, 1981). Mass mortalities of *S. droebachiensis* in the early 1980s, due to an amoebic disease, enabled the re-establishment of kelp beds (Scheibling, 1986). A new species of amoeba, *Paramoeba invadens*, was isolated from moribund sea urchins in 1983 and identified as the causative agent of this disease (Jones, 1985; Jones and Scheibling, 1985). Phylogenetic analysis of nuclear small subunit (SSU) ribosomal DNA (rDNA) isolated from moribund sea urchins in 2012 has confirmed the identity of *P. invadens* as a distinct species (Feehan et al., 2013).

Over the past 3.5 decades, recurrent outbreaks of the amoebic disease (termed paramoebiasis) have decimated sea urchin populations

* Corresponding author.
E-mail address: Robyn.Buchwald@dal.ca (R.T. Buchwald).

http://dx.doi.org/10.1016/j.jembe.2015.04.006
0022-0981/

in the shallow subtidal zone (<25 m depth) off Nova Scotia. These events are increasing in frequency with increasing sea temperatures and frequency of strong storms (Feehan and Scheibling, 2014; Scheibling and Lauzon-Guay, 2010; Scheibling et al., 2013). Paramoebiasis occurs in the late summer and early fall (August to October), around the annual temperature peak (Scheibling and Lauzon-Guay, 2010; Scheibling and Stephenson, 1984; Scheibling et al., 2013). A previous laboratory study indicated that the growth rate of *P. invadens* in culture was maximal between 15 and 20 °C (Jellett and Scheibling, 1988b), corresponding to the annual temperature peak when paramoebiasis propagates rapidly in nature (Feehan et al., 2012; Scheibling and Stephenson, 1984) and below the upper thermal tolerance limit of the urchin host (22 °C; Percy, 1973). Growth rate of the amoeba dropped sharply at 10 to 12 °C, which marks the lower thermal threshold for propagation of paramoebiasis, below which infected sea urchins appear asymptomatic and can recover (Scheibling and Hennigar, 1997; Scheibling and Stephenson, 1984). At 2 and 5 °C, *P. invadens* exhibited negative growth in culture (Jellett and Scheibling, 1988b).

The origins of source populations of *P. invadens* that infect sea urchins in Nova Scotia are unknown (Scheibling et al., 2013). To date, *P. invadens* has only been detected in the tissues of moribund sea urchins (Jellett and Scheibling, 1988b; Jellett et al., 1989). It has been assumed that *P. invadens* is unable to establish a resident population along the coast of Nova Scotia (Feehan et al., 2012; Jellett et al., 1989), where annual minimum sea temperatures in the shallow subtidal zone often drop to below 1–2 °C. Thus *P. invadens* is believed to be an alien species that is eliminated from shallow coastal waters during the winter months following a disease outbreak (Scheibling and Hennigar, 1997). A link between these outbreaks and large-scale meteorological events (hurricanes and tropical storms) suggests that the amoeba is periodically reintroduced to Nova Scotia via advective transport or vertical mixing of shelf waters with passing storms (Scheibling and Hennigar, 1997; Scheibling and Lauzon-Guay, 2010). This "recurrent introduction hypothesis" is supported by the fact that disease outbreaks do not occur every year that sea temperatures exceed the lower thermal threshold for infective paramoebiasis (Scheibling and Hennigar, 1997).

Observations of increasing minimum sea temperatures, and two instances of disease outbreaks in the absence of storms since records began (1983 and 2012) suggest an alternative (but non-exclusive) hypothesis to explain an increase in frequency of paramoebiasis in Nova Scotia (Feehan, 2015; Scheibling et al., 2013). The "resident pathogen hypothesis" holds that *P. invadens* has gained the capability to overwinter in the shallow subtidal zone, either through selective adaptation to low sea temperatures, or because the annual temperature minimum has increased. To test this hypothesis, we repeat and extend the experimental work of Jellett and Scheibling (1988b) on the temperature-dependence of growth of *P. invadens* in culture, using new cultures isolated during 2012. The chief aims were 1) to determine whether the lower tolerance limit of amoebae from natural populations has decreased over the past 3 decades, and 2) to examine the potential for survival of *P. invadens*, maintained in culture at low temperatures, following gradual warming to an optimal temperature for growth. We relate our findings to long-term records of winter temperature minima in the shallow subtidal zone (<12 m depth) to assess the potential of *P. invadens* to persist in shallow water following a disease outbreak.

2. Materials and methods

2.1. Monoxenic culturing of P. invadens

P. invadens isolate GI was isolated from the radial nerves of moribund sea urchins (*S. droebachiensis*) collected during a disease outbreak on 26 August 2012 at Gravel Island, outside of St. Margarets Bay, Nova Scotia (N 44° 29.531′, W 64° 01.691′), using the methods of Feehan et al. (2013) and Jones and Scheibling (1985). Briefly, sections of radial nerve (5 mm long), with associated radial water-vascular canal, were excised from moribund sea urchins using sterile technique and cultured onto 0.6% non-nutrient seawater (NN) agar. After 1 week at 18 °C, *P. invadens* had migrated out of the sea urchin tissue, and 1 cm^2 pieces of 0.6% agar, containing amoebae, were treated with antibiotics (10 000 IU penicillin, 10 000 μg streptomycin for 6 h) and subcultured onto 1.2% NN agar with a liquid overlay containing *Escherichia coli* as

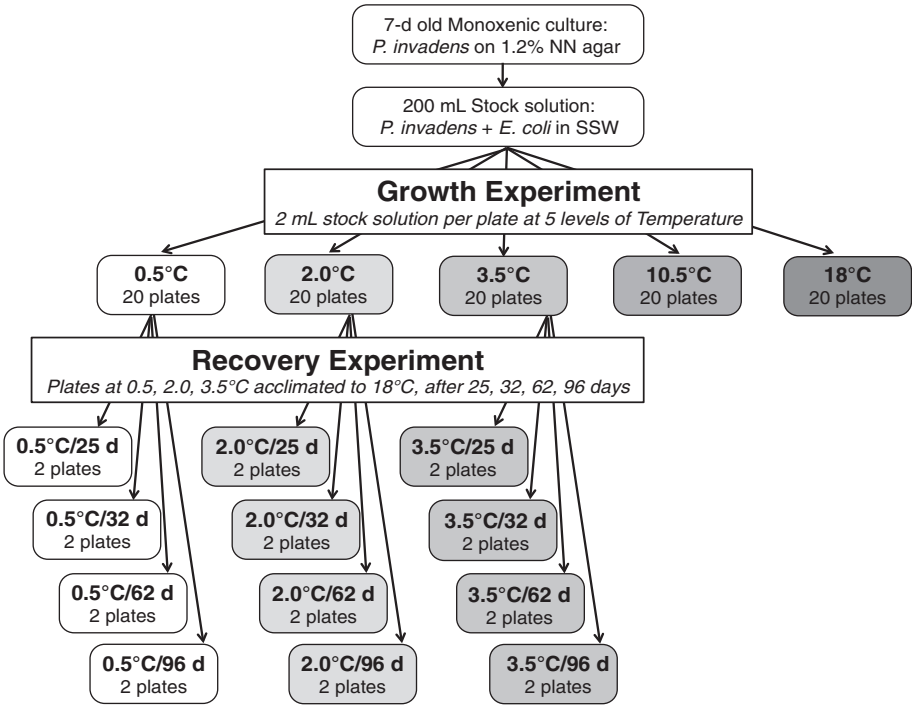

Fig. 1. Schematic of the 2 laboratory experiments conducted to examine the effect of exposure to low temperatures (0.5, 2.0, 3.5 °C) on the growth rate (growth experiment) and recovery potential (recovery experiment) of *Paramoeba invadens*.