International Biodeterioration & Biodegradation 63 (2009) 289–296

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

A laboratory assay for measuring feeding and mortality of the marine wood borer *Limnoria* under forced feeding conditions: A basis for a standard test method

L.M.S. Borges [a,b,*], S.M. Cragg [a], S. Busch [a]

[a] Institute of Marine Sciences, School of Biological Sciences, University of Portsmouth, Ferry Road, Hampshire PO4 9LY, UK
[b] Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar 4710-057 Braga Portugal

ARTICLE INFO

Article history:
Received 13 September 2008
Received in revised form
23 October 2008
Accepted 23 October 2008
Available online 1 February 2009

Keywords:
Laboratory trials
Screening tests
Limnoria quadripunctata
Test conditions
Wooden materials

ABSTRACT

Quick simple testing methods are needed to evaluate alternative wood materials for marine construction because traditional borer resistant materials are becoming scarce or are no longer permitted due to concerns over environmental emissions of preservatives. Laboratory tests can provide species-specific information on rates of wood biodeterioration by wood borers under optimum conditions, in contrast to field trials where more than one borer species may be present and conditions are variable.

The methodology described herein relies on the assumption that faecal pellet production rate in limnoriids must match feeding rate quite closely. Thus, the number of faecal pellets produced by individual specimens of *Limnoria quadripunctata*, while feeding on a non-durable and non-toxic wood species – *Pinus sylvestris* sapwood – in different test conditions, was monitored over a period of 15 days. Mortality and moulting were also registered. Several variables likely to affect survival and feeding rates were investigated in order to optimise the test conditions. Temperature and salinity regime affected both survival and feeding rates while moulting cycle affected feeding rates.

The optimisation of this test methodology aims to provide the basis for a standard laboratory test with the wood-boring crustacean *Limnoria*.

1. Introduction

Wood used in maritime construction is subject to attack by marine wood borer molluscs and crustaceans. Nevertheless, it has been used as a construction material for centuries due to properties such as resilience, favourable strength-to-weight ratio, relatively low energy costs of production and renewability (Cragg, 1996). Durable tropical timbers are widely used in marine construction but there is no guarantee that supply of these can continue to satisfy demand. Therefore, lesser-utilised wood species (LUS) needed to be tested for their suitability for the marine environment (Borges et al., 2008a). In addition, non-biocidal wood treatments, which modify the chemistry of structural components of wood, need to be tested for their resistance to wood-boring organisms before being introduced to the marine environment (Borges et al., 2004; Borges, 2007).

Marine trials generally need to extend over a number of years in order to generate meaningful results (BS EN 275, 1992), which makes them expensive and time consuming. Therefore, there is a need for quick and cheaper testing methodologies. As an alternative, laboratory tests have been developed, to speed up the evaluation of LUS (Borges et al., 2003; Rosenbusch et al., 2006) and modified wood (Borges et al., 2004). Indeed certain treated timbers were found to be more severely attacked in laboratory than in field conditions by creating optimum conditions in the laboratory for test organisms (Cookson, 1996; Cookson and Woods, 1995; Borges, 2007).

Limnoriids have been successfully cultured since the 1930s for use in laboratory trials as test organisms (Becker, 1944; Cookson, 1990; Kühne and Becker, 1970; Parrish et al., 1983). Due probably to its more restricted distribution, *Limnoria quadripunctata* has not been as extensively studied as *Limnoria lignorum* (Henderson, 1924; Johnson, 1935; Sømme, 1940) or *Limnoria tripunctata* (Beckman and Menzies, 1960; Johnson and Menzies, 1956; Menzies, 1951) and perhaps because of that has been less used as a test organism. For a long time it was thought this species was a lesser threat for wood than the other two more common species. However, Barnacle et al. (1983) presented evidence from field surveys that *L. quadripunctata*

* Corresponding author. CBMA (Centre of Molecular and Environmental Biology)/Department of Biology/University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. Tel.: +351 253601518.
E-mail addresses: luisa.borges@port.ac.uk, luisaborges2000@yahoo.co.uk (L.M.S. Borges).

0964-8305/$ – see front matter
doi:10.1016/j.ibiod.2008.10.007

can cause considerable destruction in untreated wooden structures and even in wood treated with chromated copper arsenate (CCA). Furthermore, in optimum conditions for the species, attack by *L. quadripunctata* was not significantly less than attack by *L. tripunctata* (Cookson, 1990), the latter usually considered to be the most destructive limnoriid species.

Testing methodology for evaluating the efficacy of wood preservatives against decay fungi and wood-boring beetles is well developed with established standard test methods for both categories of wood degraders (see for example BS EN 20-1:1992). For marine borers on the other hand, field-testing methods are recognised (e.g. BS EN 275:1992), but there is no laboratory test equivalent to those used for wood-boring insects. This means that the normal test strategy for novel methods of wood protection consisting of laboratory screening, standard laboratory testing then field trials is not available for methods applicable in the marine environment. For such a test to be useful it must meet requirements of reproducibility and optimal conditions for the test organism. Thus, to optimise conditions for the use of *L. quadripunctata* as a test organism in laboratory assays, it was important to investigate a number of environmental and animal-related variables likely to affect both their survival and feeding. Three variables have been reported as affecting boring activity of limnoriids: water temperate (Kühne, 1971; Menzies, 1957), salinity (Eltringham, 1961) and light (Isham et al., 1951). Most of these studies were conducted in field trials with several wood-borer species present and variable conditions (e.g. Eltringham and Hockley, 1958). Thus it was important to test these variables under controlled laboratory conditions, in order to identify the optimum conditions for *L. quadripunctata* to be used as test organism.

Direct observations of effects of toxicants on animals isolated from wood were conducted with a wide range of organic and inorganic compounds in solution (White, 1929; Hochman and Vind, 1966) and with insecticides by contact (Rutherford et al., 1979). However, most currently used methods of wood protection act by interfering with the digestive process, so testing needs to permit feeding on the wood material to be tested. Laboratory tests using colonies of *Limnoria* with the outcome assessed by measuring wood loss or rating on an arbitrary scale have required periods of exposure ranging up from 3 to 24 months (Kühne, 1968; Richards and Webb, 1975; Cookson, 1999). Shorter-term tests with a known number of test organisms for reproducibility are desirable. The current study aims to satisfy these requirements and provide the basis for a standard laboratory test with the wood-boring crustacean *Limnoria*.

2. Materials and methods

2.1. Laboratory cultures of L. quadripunctata

Specimens of *L. quadripunctata* Holthuis were obtained from infested beach defence works at Southsea, Portsmouth, UK. Water temperature registered in the area ranges from 4.7 °C to 22.5 °C (Watson, unpublished data). The infested wood was then used to establish a culture, at the Institute of Marine Sciences, in indoor tanks at a constant temperature of 20 ± 1 °C, with seawater of salinity of approximately 33 PSU obtained directly from Langstone Harbour, Portsmouth, UK.

Prior to the experimentation, the wood was wrapped in kitchen cloths for 24 h as recommended by L.J. Cookson. The anoxic conditions created induced the limnoriids to leave their burrows, permitting them to be easily removed with little or no damage. To make sure that only undamaged animals were used in the experiments, they were transferred with a fine sable brush or fine forceps to a smaller box with seawater and aerated for 24 h. This allowed any damage resulting from the extraction technique to become apparent. Only undamaged specimens were used. Their identification was based upon the descriptions in Menzies (1957, 1959) and Kühne (1971). The specimens were checked for the presence of folliculinids on the telson and only the ones free of folliculinids (ciliate epibionts on the pleotelson of limnoriids) were used in the experiments as the presence of these organisms in *Limnoria* is associated with lowered feeding rates in the latter (Delgery et al., 2006).

2.2. Experimental set-up

Test sticks measuring 20 × 4.5 × 2.5 mm, were prepared from sapwood of *Pinus sylvestris* to be used as food source. The sticks were leached for a week in a 500-ml beaker of seawater prior to the experiments.

Cell-culture boxes with 12 wells measuring 20 mm in diameter were used in all experiments to test animals individually. A single specimen of *L. quadripunctata* was placed in each well, together with a test stick and 4 ml of seawater with a salinity of 31–34 PSU and covered with a lid to avoid evaporation (Fig. 1).

Feeding rate was measured indirectly by counting the number of faecal pellets produced. In the light trial 24 replicates were used and the number of faecal pellets was counted after one and two weeks. In the temperature trial 12 replicates were used and the number of faecal pellets was counted every third day. In the salinity trial 30 replicates were used and the number of faecal pellets was also counted after one and two weeks.

Cell-culture boxes were covered with aluminium foil to exclude light and stored in temperature-controlled chambers at the temperatures at 20 ± 1 °C. Variations to this general conditions were introduced in each of the tests (please see below). All tests were conducted under static seawater conditions. The number of moulting and dead animals as well as the position of the animals in the wood (inside or outside) was recorded. After recording the number of faecal pellets, the animals and the wood were then transferred to clean chambers with fresh seawater kept at the same temperature.

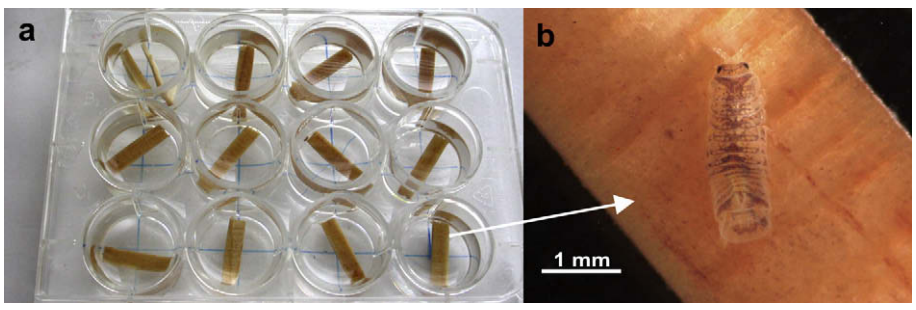

Fig. 1. (a) Cell-culture box used in temperature trials. (b) *Limnoria quadripunctata* on test stick.