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Research paper

Improved methodology for measuring pore patterns in the benthic foraminiferal genus *Ammonia*

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ABSTRACT

Benthic foraminiferal pores are considered to play an important role in facilitating the gas exchange between the organism and the environment, with pore size and density supposed to be related to gas exchange intensity. Recent studies have therefore attempted to establish relationships between pore patterns and redox conditions, such as bottom water oxygen and nitrate concentrations. However, a prerequisite for such an attempt is the development of a practical and reliable methodology for measuring pore patterns. The aim of this study is to present a semi-automated pore measurement method for *Ammonia*, a dominant taxon of temperate coastal environments that are increasingly affected by seasonal hypoxia (bottom water oxygen concentration < 63 μM). The approach is based on image analyses of a measurement frame positioned on SEM images with 1000× magnification. Statistical analyses show that the surface area of the pores in the frame has a normal distribution. Therefore, a mean pore surface area can be used to describe the pores in the measurement frame. We observed small but significant ontogenetic changes in pore density (number of pores per frame) and pore surface area. Accordingly, it seems preferable to limit pore measurements to size windows on chambers representing the same ontogenetic stage.

In order to demonstrate the efficiency of the method, we applied it in two case studies. Firstly, a study of living *Ammonia* in Lake Grevelingen (Netherlands) revealed a clear difference in pore patterns between three studied stations characterised by different seasonal bottom water oxygenation patterns. Secondly, a sediment core from the same site clearly showed the presence of two morphotypes of *Ammonia*; one with numerous, small pores and the other with fewer but much larger pores, resulting in a higher porosity (larger part of the test covered by pores). Since the man-made closure of Lake Grevelingen in 1971, the latter morphotype has progressively replaced the former one. Finally, a summary of the measurements on 870 specimens with both pore patterns shows a strong relationship between pore density and pore surface area, suggesting a physical control of the interaction between these two parameters.

1. Introduction

Studies focusing on pores in foraminifera are relatively scarce. Hofker (1950) was probably the first to study foraminiferal pore patterns in a semi-quantitative way. He described morphological differences between species of the same genus from different geologic times (Hofker, 1950, 1951). Bé (1968) studied pore characteristics of various recent planktonic foraminiferal species, and found that species of specific climate zones have similar pore patterns. Pores are formed at an early stage during chamber formation, giving rise to discussion on their role in the calcification process (Banner and Williams, 1973; Berthold, 1976; Hemleben et al., 1977; Spero, 1988). For example, it has been suggested that they can serve as a site of osmotic exchange between the exterior and interior of a newly formed chamber (Banner and Williams, 1973). More importantly, pores of benthic foraminifera (BF) are supposed to be related to gas exchange, and specifically oxygen uptake, partly because of the concentration of mitochondrial clusters and

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ectobionts in the proximity of the pore openings (Leutenegger and Hansen, 1979; Bernhard et al., 2010). This led to the hypothesis of increased porosity in taxa and/or populations living in low oxygen environments (e.g., Gary et al., 1989; Sen Gupta and Machain-Castillo, 1993). Perez-Cruz and Machain-Castillo (1990), for example, observed that *Hanzawaia nitidula* had larger and more numerous pores in an East Pacific Oxygen Minimum Zone (OMZ) than in more oxygenated waters. Recently, a significant correlation has been shown between pore density and bottom water oxygen (BWO) and/or nitrate concentration in several deep-sea species (Glock et al., 2011; Kuhnt et al., 2013). However, important interspecific differences in pore parameters also exist (Gooday and Alve, 2001). In fact, molecular data have shown that certain pseudocryptic species show clear differences in porosity, e.g., in *Ammonia* spp. (Holzmann and Pawlowski, 1997; Hayward et al., 2004), *Cibicides/Cibicidoides* (Schweizer et al., 2009), as well as in the planktonic taxa *Orbulina universa* (de Vargas et al., 1999; Morard et al., 2009) and *Globigerinella siphonifera* (Huber et al., 1997). Therefore, although some phenotypic plasticity appears to exist, porosity is not only dependent on the environment, but is also genetically encoded.

In coastal environments of temperate climate zones, representatives of the *Ammonia tepida* complex are often the dominating benthic foraminiferal communities. They are capable of living in extremely variable environments and tolerating diverse biological stress factors (Murray, 2006). Therefore, numerous studies addressed their morphology (e.g., Bermudez, 1952; Banner and Williams, 1973; Poag, 1978; Jorissen, 1988), life strategies (e.g., Bradshaw, 1957; Geslin et al., 1998; Stouff et al., 1999; Moodley et al., 2000; Thibault De Chanvalon et al., 2015; Cesbron et al., 2016) and genetic variability (e.g., Holzmann and Pawlowski, 1997; Hayward et al., 2004; Schweizer et al., 2011; Saad and Wade, 2016). The morphological *A. tepida* complex includes at least three different pseudocryptic species in Europe as shown by molecular studies (Hayward et al., 2004). A morphological reexamination of specimens from known phylotypes allowed finding slight differences (making them pseudocryptic instead of cryptic species), but it is very difficult to discriminate these species solely based on the morphology of the test (e.g., Hayward et al., 2004). Therefore, the name *A. tepida* is used here, knowing that it designates a species complex including several pseudocryptic species. Concerning a potential relationship with bottom water oxygenation, Moodley and Hess (1992) found that *A. tepida* from the southern North Sea (determined as *A. beccarii*) survives anoxic (no measurable BWO concentration) periods and exhibits higher porosity under low oxygen conditions than under normoxic conditions in laboratory experiments. Kitazato and Tsuchiya (1999) conducted similar culture studies and found that the pore diameter increased with lower dissolved oxygen concentrations. *Ammonia tepida* (determined as *A. parkinsoniana*) assemblages dominate BF faunas on shelf environments in the Gulf of Mexico with seasonally hypoxic bottom waters (BWO concentration < 63 μM; Rabalais et al., 1996, 2002; Sen Gupta et al., 1996). Recently, it has been confirmed by culture experiments that *A. tepida* is able to survive and calcify under hypoxic as well as anoxic conditions (Geslin et al., 2014; Nardelli et al., 2014).

In many coastal areas worldwide low BWO concentration is presently intensified by increasing eutrophication, as well as by global warming, which leads to reduced dissolved oxygen solubility and enhanced water column stratification. Ecological consequences are increased duration, extension and intensity of hypoxia, inducing severe stress on benthic faunas (Diaz and Rosenberg, 2008; Riedel et al., 2016). In view of these findings, it becomes urgent to further analyse the relationship between foraminiferal pore patterns and sea floor redox conditions, and to explore the potential of pores as a palaeoceanographic proxy.

The aim of our study was to develop a practical, reliable and easily reproducible method for quantitatively describing pore patterns in *Ammonia*. In this paper, we will first discuss previously used methodologies for pore measurements (e.g., Wiles, 1965; Moodley and Hess, 1992; Glock et al., 2011; Kuhnt et al., 2013, 2014; Weiner et al., 2015). In most cases these methods are adapted to a certain species or context, and are not necessarily suited for measuring pores in *Ammonia* and genera with a similar morphology and pore pattern. Most of these methods are not efficient, when measuring large quantities of specimens. The semi-automated method presented here has been specifically designed for *A. tepida* and species with a similar morphology. We will illustrate the efficiency and reliability of this new method in two case studies.

2. Methodological developments

2.1. Overview of previously published methodologies for measuring pore patterns in foraminifera

Pore analysis has been applied to foraminiferal tests since the 1950s (Hofker, 1950, 1951). Wiles (1965) presented a detailed description of his methodology. He used the inner surface of crushed, fossil shells from a single planktonic species (*Globigerina eggeri*), oriented parallel between two glass slides, on which he measured the pores with a petrographic microscope. At a magnification of 270×, a square frame with a length of 45 μm was used to delimit the area for pore measurements and to calculate the pore density, defined as the number of pores per surface area (see Table 1 for definition of pore parameters). In his pore analyses, no correction was made for partial pores, touching the border of the frame. For each specimen, the pores of at least three fragments were counted at least three times and an average of twenty specimens was considered representative for the pore characteristics of the analysed species (Wiles, 1965). Bé (1968) followed this procedure for analysing the pores of various living planktonic species but used 600× magnification and a square frame with a length of 25 μm. Besides pore number and density, the pore diameter was reported as well, allowing the calculation of the porosity (the percentage of frame area occupied by pores, Bé, 1968; Bé et al., 1973).

The studies of Frerichs and co-workers (Frerichs et al., 1972; Frerichs and Ely, 1978) used a similar approach for recent planktonic species (frame area = 1240 μm^2, 450× magnification). Moreover, Frerichs et al. (1972) specified that only the last chamber of the largest specimen of each species was used. On the basis of a comparison of mean, maximum and minimum diameters of single pores, they concluded that in all five analysed species the minimum pore diameter, and therefore the porosity, decreases with increasing distance to the equator (Frerichs et al., 1972). Earlier, Lutze (1962) had indicated that the precision of pore measurements made under a binocular microscope is limited by difficulties in adjusting the focus on the pore openings. More recently, for benthic foraminifera, it has become usual to measure the pores on the outside of the test, using SEM images (e.g., Moodley and Hess, 1992). Holzmann and Pawlowski (1997) and Hayward et al. (2004) used pore analyses of SEM images together with molecular analyses to discriminate species of the genus *Ammonia*. In Hayward et al. (2004), measurements were systematically performed on 10 pores in the penultimate chamber, closest to the junction of spiral and chamber sutures (with the last chamber). Contrasting to the approach using a frame, Glock et al. (2011) counted all pores of one side of tests of *Bolivina spissa* on SEM images. This number was considered as half of

Table 1
Glossary of terms related to pore measurements used in this study.

Number of pores [ ]	Total number of pores (Np) automatically counted in the measurement frame, after manual correction for partial pores, double pores and pores of exceptionally small size
Pore density [Np/μm^2]	Number of pores per surface area of measurement frame (the method presented here uses a frame with a surface area of 562 μm^2)
Pore area [μm^2]	The mean surface area of all pores, calculated as the total surface area occupied by pores, divided by the corrected number of pores, expressed in μm^2
Porosity [%]	Percentage of the surface in the measurement frame covered by pores.