Spatial distribution of *Alitta virens* burrows in intertidal sediments studied by axial tomodensitometry

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Relationships between sediment characteristics and the spatial organization of biogenic structures remain poorly understood, albeit important for characterizing bioturbation impacts and small-scale ecological patterns. Using axial tomodensitometry (CT-scan) and core sectioning, we studied the spatial distribution of Alitta virens *burrows in sediment cores from two mud-flats with different degrees of exposure along the St Lawrence Estuary, Canada. A variety of burrow morphologies was observed at both sites, with most being I-shaped. Most values measured (organic matter content, mean tomographic intensity, the number and diameter of burrow shafts and the percentage of space they occupy per transverse section) covaried with depth. The more sheltered site had higher organic matter and mud contents, and lower average tomographic intensity values. The spatial distribution of burrow shafts also differed between sites, with the more sheltered site having a higher number of burrow shafts and percentage of biogenic space in the upper sediment column, as well as a greater volume of bio-genic structures that were connected to the surface (although intra-site variability was great). Along the horizontal plane and at various depths within sediments, burrow shafts were more randomly distributed at the exposed site, compared to a more even, maximal spacing at the sheltered site. In addition to finding differences in burrow organization at two spatial scales, we found significant correlations between tomographic intensity and (1) the number of burrow shafts, (2) biogenic space and (3) organic matter content. CT-scan data, including tomographic intensity, are useful for examining and comparing biogenic structures in sediment cores.*

Keywords: *Alitta virens*, burrows, axial tomodensitometry, bioturbation, aggregation index, CT-scan

INTRODUCTION

Macrobenthic organisms have evolved different strategies for obtaining resources, eliminating wastes, and taking part in various biotic interactions within sedimentary habitats. One such strategy consists of establishing long term or semi-permanent burrows through which the transport of water, associated particles and solutes can be induced via the venti-lation activities of the resident organism. Burrow formation and irrigation can alter sediment structure at different scales (Aller & Yingst, 1978; Mermillod-Blondin *et al.*, 2004; Zorn *et al.*, 2010) and can result in an enhancement or inhibition of nutrient and gas fluxes within the sediment column (Kristensen, 1984, 1985; Krantzberg, 1985; Gerino *et al.*, 2003). Burrow walls form important microhabitats with specific microbial and meiofaunal communities (Reise, 1981; Commito & Shrader, 1985; Mermillod-Blondin *et al.*, 2004) that can modify biogeochemical processes in adjacent sedi-ments (Kristensen, 1985; Papaspyrou *et al.*, 2006).

Burrows can consist of complex structures with multiple branches (or shafts) and openings to the sediment surface (Miron *et al.*, 1991a, b; Davey, 1994; Gingras *et al.*, 2008). Although there is no direct evidence that the three-dimensional (3D) organization of such structures can influ-ence bioturbation or bioirrigation processes, changes observed during the establishment of burrows suggest a role for burrow form (Kristensen *et al.*, 2012). For example, the polychaete *Alitta virens* Sars, 1835 (synonyms *Nereis virens*, *Neanthes virens*, Bakken & Wilson, 2005) constructs burrows of differ-ent shapes (I, U, J and Y) and complexity (from simple to multi-branched forms) (Reise, 1981; Miron *et al.*, 1991b; Herringshaw *et al.*, 2010). Studies of this species have shown that over the course of burrow formation and maintenance, the total sedimentary oxygen uptake (Kristensen, 1984, 1985; Michaud *et al.*, 2005) and levels of particle transport induced by the worm's activity will vary (Michaud *et al.*, 2005; Piot *et al.*, 2008; Kristensen *et al.*, 2012).

The role of burrow form is generally not taken into account when considering the functional role of burrowing organisms, mainly due to the difficulty in obtaining 3-D structure data in a sediment column. However, data regarding the shape, volume and surface area of burrows are likely to be important for the study and calibration of bioturbation models (François

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et al., 1999, 2002; De Montety *et al.*, 2003), for relating organism behaviour to flux rates, and for understanding the functioning of both current and past sedimentary ecosystems (Gingras *et al.*, 2008; Zorn *et al.*, 2010; Kristensen *et al.*, 2012). Further, burrow form can be affected by sediment grain size, elasticity, porosity and degree of compaction (Brenchley, 1982; Miron & Desrosiers, 1990; Daschtgard *et al.*, 2008), so the consideration of both sedimentary and burrow characters is desirable when evaluating the functional role of burrow forming organisms.

Although researchers have described the three-dimensional structure of burrows formed in aquaria (Miron *et al.*, 1991b; Herringshaw *et al.*, 2010), and in the field using resin casts (Risk *et al.*, 1978; Dworschak, 1983; Davey, 1994; Butler & Bird, 2007), we are not aware of any study of the spatial organization of multiple complex burrows *in situ* and within different types of sediments. Axial tomodensitometry (CT-scan) is a useful and rapid approach for the visualization and biovolume quantification of burrows and other biogenic structures within sediment cores (De Montety *et al.*, 2003; Mermillod-Blondin *et al.*, 2003; Michaud *et al.*, 2003; Rosenberg *et al.*, 2007, 2008); this technique could be used to investigate variability in the spatial organization and biovolume of burrows at different scales. Here, we use CT-scanning to describe and compare burrow assemblages within intertidal sediments dominated by *A. virens*. Burrow metrics (number, volume, diameter, degree of aggregation) are compared at two different scales: within and between intertidal mudflats separated by 20 km and experiencing different environmental conditions (exposed vs sheltered). We hypothesize that: (1) significant differences in burrow characteristics exist between sites, but that (2) there are no significant differences within a given site, suggesting that the functional importance of *A. virens* in different intertidal sites can vary. Also, we evaluate the usefulness of the CT-scan metric tomographic intensity (TI) as a predictive parameter, given that TI is influenced by several factors such as grain size, sediment compaction and composition, and biogenic occupation (Crémer *et al.*, 2002; De Montety *et al.*, 2003; Mermillod-Blondin *et al.*, 2003; Michaud *et al.*, 2003).

Alitta virens burrows are good models for such a study: they are long-term burrows, consolidated by mucus (Gingras *et al.*, 2008; Herringshaw *et al.*, 2010; Zorn *et al.*, 2010), and of variable complexity (Miron *et al.*, 1991b; Gingras *et al.*, 2008; Herringshaw *et al.*, 2010). Each *A. virens* individual establishes its own burrow, and there is no connection between the galleries of different individuals (Miron *et al.*, 1991b). *Alitta virens* are common and abundant members of boreal *Macoma balthica* Linnaeus, 1758 communities (Desrosiers *et al.*, 1980; Desrosiers & Brêthes, 1984), with estimated abundances reaching close to 500 individuals m^{-2} in the intertidal zone of the St Lawrence Estuary (Miron & Desrosiers, 1990).

MATERIALS AND METHODS

Study sites

Sediment core sampling occurred in June 2005, on the northern shore of the Chaleur Bay, on the Gulf of St Lawrence, Canada (Figure 1). Two intertidal mudflats, adjacent to salt marshes and separated by approximately 20 km of coastline, were chosen: (1) the exposed site, in Paspébiac (P), was

periodically connected to the bay by a narrow channel and submitted to tidal exchange and little riverine input; and (2) the sheltered site, at St-Siméon(S), was a non-vegetated patch within a salt marsh sheltered by a sand bar and receiving greater river effluents (Renaud, 2000). At both sites, the benthic macrofauna is dominated by the polychaete *Alitta virens* (personal observation during slicing of the cores), the only resident species that constructs long term burrows.

Core sampling and sediment analysis

At each site, four samples, separated from each other by approximately 1–3 m, were obtained haphazardly using aluminium push cores (diameter: 10 cm) from patches of mudflat that appeared homogeneous and had apparent burrow apertures. Cores were pushed by hand until a high degree of resistance was felt; as a result, core length varied between 20 and 30 cm between sites. Cores were sealed at the bottom with a rubber cap and duct tape and at the top with a rubber-rimmed aluminium screw-top seal. All cores were kept upright and refrigerated at 4°C until they were scanned (up to seven days after collection) and then extruded. We assume that burrow structures were not significantly altered between core collection and CT-scanning because of the partial drainage of water that resulted from the sampling approach (particularly in the burrow lumina; this probably led to individuals taking refuge at the bottom of their burrows), and because refrigeration likely limited worm activity (Ouellette *et al.*, 2004). We cannot confidently report on the number of organisms that were alive within the cores at the time of collection because of the delay between sampling and core extrusion following CT-scanning (due to transportation between sampling site and the CT-scanning facility, and availability of the instrument).

After scanning, sediment grain size was determined for samples obtained at the surface, middle, and bottom of each core, using a Beckman LS 13 320 Coulter counter (size range: 0.04–2000 μm) and Gradistat software in Excel®. Sediment grain sizes were divided into three categories: gravel (>500 μm), sand (63–500 μm) and mud (<63 μm).

The percentage organic matter (OM) content in sediments was determined by loss of dry mass following ignition (48 h at 450°C) as in Heiri *et al.* (2001). Sediment samples for organic matter determinations were taken from the sediment surface and at 2 cm intervals for the entire length of each core.

CT-scanning and 3-D reconstructions

Cores were scanned along their entire length at 140 kV using a Siemens Somatom Volume Access scanner at the Institut National de la Recherche Scientifique, Eau Terre Environnement, Quebec City, Canada. The Siemens scanner uses 26,000 spatial projections to reconstruct a series of consecutive 2D sections of the scanned object with a spatial resolution of at least 0.1 mm. For each scanned volume unit (voxel), the tomographic intensity (TI) is measured and given in Hounsfield units (Hounsfield, 1973), where the TI represents the absorption coefficient of X-rays within the voxel relative to the absorption coefficient of water, and is related to the density, atomic number and radiation–matter principles of all particles contained in the voxel (Duliu, 1999). The TI of each voxel is then represented by a grey scale of 4096 values on a 2-D matrix of 512 × 512 pixels
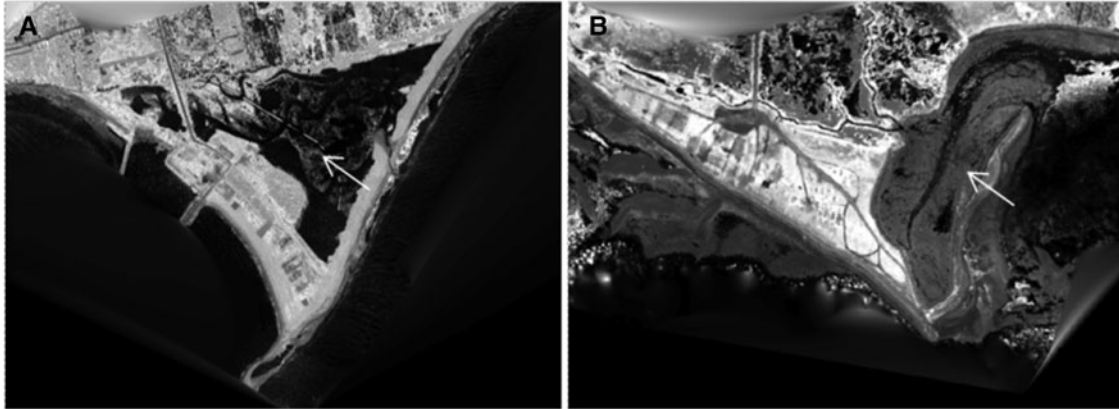
Fig. 1. Location of the two sampling sites, on the southern shore of the Gaspé peninsula, Quebec, Canada. (A) Paspébiac (48°2′0.02″N, 65°15′0.02″W); (B) St-Siméon (48°4′0.02″N, 65°34′0.02″W). Arrows indicate sampling sites on Lidar images of the two salt marshes.

(Figure 2). In scanned sediment cores, the TI depends mostly on the density of particles and the presence of air or water filled spaces within each voxel (Michaud *et al.*, 2003). Grain size, mineral composition and compaction of sediments also influence TI values (Boespflug *et al.*, 1995; Michaud *et al.*, 2003).

Consecutive transverse sections were scanned at a thickness of 1 mm for each core. From this series, 3-D reconstructions of biogenic structures were made using the Siemens software and the freeware OsiriX v.3.6, as in Dufour *et al.* (2005).

CT-scan image analysis

For every fifth transverse section, the number of all air or water filled structures (with TI values <0 HU) measuring >2 mm in diameter (to exclude meiofauna and juveniles) and appearing on more than three consecutive sections was determined. These structures are hereafter referred to as burrow shafts: because of the complex form of *Alitta virens* burrows, an individual burrow can have more than one shaft on a 2-D core section (Figure 2). Their mean diameter and central spatial coordinates were recorded, and the average TI of all materials within the core liner was determined by selecting the core area as a region of interest in ImageJ® (http://rsbweb.nih.gov/ij/; Abramoff *et al.*, 2004). From this region of interest, the surface area was obtained, and the percentage of space occupied by biogenic structures was calculated, as in Dufour *et al.* (2005). Measurements
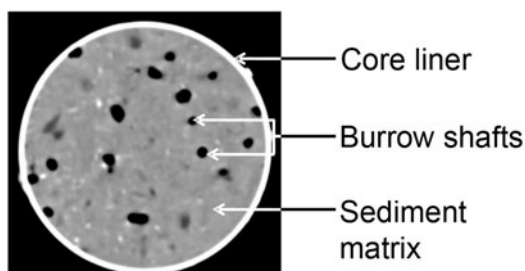

Fig. 2. Transverse CT-section of a sediment core (10 cm in diameter). Tomographic intensity, number and diameter of burrow shafts, percentage biogenic space, and spatial coordinates were determined or calculated from such images using ImageJ.

began 5–10 mm below the sediment surface because surface irregularities would have led to biases in the uppermost sections.

For each fifth section, the number of burrow shafts, their central coordinates and the section area were used to calculate the R index (Clark & Evans, 1954). This index, generally used to determine the level of spatial organization of individuals of a single species (such as trees in a quadrate), has previously been used to determine the spatial structure of burrow apertures of polychaetes and terebellids (Anderson & Kendziorek, 1982; Miron *et al.*, 1991b). The R index is a nearest-neighbour based estimation of the spatial distribution of points along a horizontal plane, on a scale from 0 (maximal aggregation) to 2.14 (maximal spacing), with values of 1 representing random distributions (Clark & Evans, 1954). We treated burrow shafts as points instead of circles; this should not influence the results since shaft diameters (average: 3.5 mm) were less than half the expected mean nearest-neighbour distance (Simberloff, 1979). The R index was determined only for sections where the number of burrow shafts was >7, as recommended by Andrew & Mapstone (1987); this represented 72 measurements in total. Significant departures from spatial randomness were tested for each R determination as in Clark & Evans (1954).

Two burrow volume determinations were made for each core: (1) the total volume of all biogenic space; and (2) the volume of those structures connected to the sediment surface (that is, excluding relict structures, as defined by Rosenberg *et al.* (2007)). Using ImageJ® and the plug-in Object counter 3-D, all biogenic structures were identified, and their volumes were determined independently and then combined to represent the total burrow volume (TBV). To quantify surface-linked burrow volume (SLBV), only the structures that reached the sediment surface were considered, and their volumes were summed for each core. *t*-tests were performed for site comparisons after verifying homogeneity of variance using Levene tests.

Statistical analyses

Given that all variables (number of shafts, diameter of shafts, percentage biogenic space, percentage organic matter, TI), with the exception of R, varied with depth, analyses of covariance (ANCOVAs) were used to test the effect of the site (fixed

factor, P and S) and cores nested within sites (random factor, four cores per site) with depth as the covariate. To account for differences in total core length, all ANCOVAs were run to an equivalent depth (115 mm). Normality was tested on the residuals using the Shapiro–Wilk test, and was observed for only two variables (diameter of shafts and TI). When a transformation was possible and met the normality assumption, it was applied. However, ANCOVAs are robust to small departures from normality when sample size is high and the design is balanced (Underwood, 1997). Plotting residuals against adjusted group means was used to check the assumption of homogeneity of variances (Quinn & Keough, 2002). Outliers (one for % biogenic space, one for % organic matter, one for tomographic intensity, three for diameter of shafts and four for % biogenic space) were removed to reach the assumption.

To investigate any relationships between variables (number of shafts, diameter of shafts, percentage biogenic space, percentage organic matter, TI, and R), Pearson correlations were calculated, for the top 115 mm, with data from all

cores combined. Also, site-specific Pearson correlations were calculated for TI and OM, because the site effect was significant.

RESULTS

Sediment characteristics

We obtained longer cores from Paspébiac (140–280 mm) than from St-Siméon (120–160 mm). Sediments from both mudflats lacked gravel, and contained proportions of sand and mud that varied within and between cores (Figure 3A). On average, there was a greater proportion of mud in cores from St-Siméon (94%) than in cores from Paspébiac (58%); at the latter site, sand tended to be more prevalent in deeper parts of the core. The shorter cores from St-Siméon were more homogeneous, containing mostly mud at all stations and depths.

Sediment organic matter content covaried with depth at both sites (Table 1). Organic matter content was greater in cores from St-Siméon (mean ± SE: 10.84 ± 0.61%) than in cores from Paspébiac (mean ± SE: 5.09 ± 0.62%); ANCOVA results show significant intra- and inter-site variability (Figure 3B; Table 1).
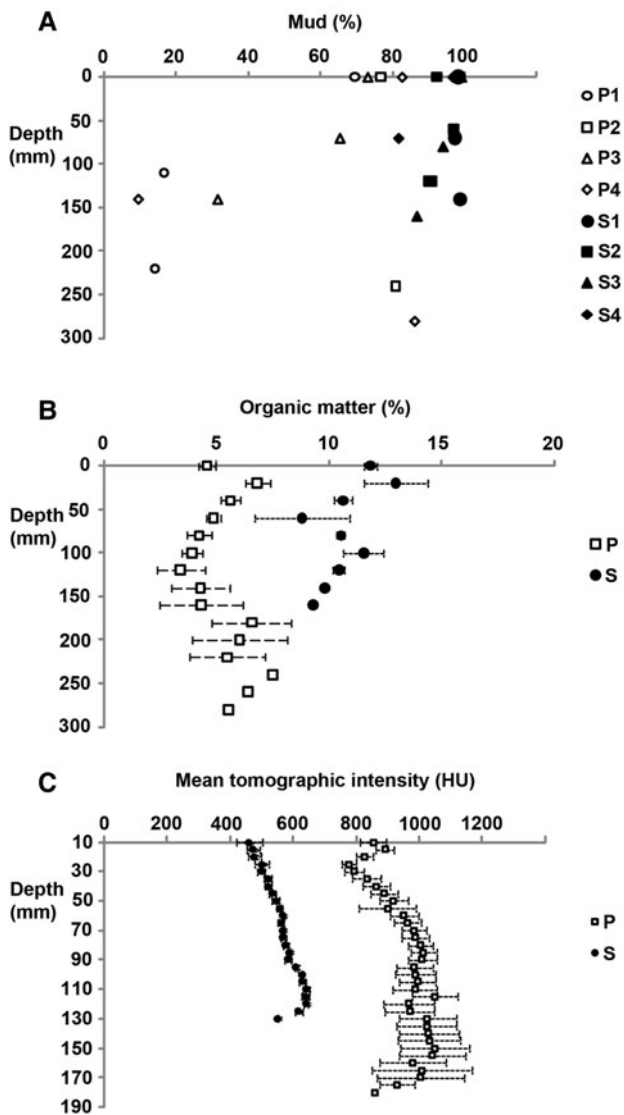

Fig. 3. Vertical distribution of (A) mud content; (B) sediment organic matter content; (C) mean tomographic intensity in cores sampled from Paspébiac (P) and St-Siméon (S). Values in (B) and (C) are averages ± SE of data from four cores per site.

Table 1. Summary of analyses of covariance with depth as covariates showing the effect of (a) % organic matter, (b) tomographic intensity, (c) number of burrow shafts, (d), diameter of burrow shafts (e) % biogenic space, and (f) R (aggregation index).

Source of variation	df	MS	*F*-value	*P*
(a) % organic matter[1]				
Site	1	475.997	110.127	<0.0001
Core (site)	6	4.330	2.823	0.0227
Depth	1	31.411	20.476	<0.0001
Error	38	1.534		
(b) Tomographic intensity				
Site	1	6.21×10^6	77.605	0.0001
Core (site)	6	8.67×10^4	37.284	<0.0001
Depth	1	6.03×10^5	259.210	<0.0001
Error	173	2.33×10^3		
(c) Number of burrow shafts				
Site	1	28.961	0.551	0.4859
Core (site)	6	52.661	3.567	0.0024
Depth	1	4104.010	278.012	<0.0001
Error	169	14.762		
(d) Diameter of burrow shafts				
Site	1	0.366	0.139	0.7227
Core (site)	6	2.670	7.643	<0.0001
Depth	1	4.838	13.849	0.0003
Error	164	0.349		
(e) Square root (% biogenic space)				
Site	1	4.283	2.167	0.1913
Core (site)	6	1.981	4.193	0.0006
Depth	1	47.564	100.656	<0.0001
Error	169	0.473		
(f) R (aggregation index)[2]				
Site	1	0.728	5.657	0.0543
Core (site)	6	0.131	3.278	0.0046
Depth	–	–	–	–
Error	158	0.040		

[1] the interaction between depth and site was significant; all sources of variation involving depth were removed because they were not significant.

The average TI of CT sections increased (and covaried significantly) with depth at both sites (Figure 3C; Table 1). Depth-specific TI values were higher and more variable on sections from the Paspébiac cores (mean $\pm$ SE: 947 $\pm$ 53 HU) than on those from St-Siméon (mean $\pm$ SE: 560 $\pm$ 18 HU); despite high intra-site variability, the site effect was significant (Table 1).

Burrow shape and volume

Entire burrows were revealed through CT-scan reconstructions of each core (Figure 4). We saw no evidence of burrow collapse. Despite variable core lengths, most burrows did not extend deeper than the length of the core. Burrows took various shapes: I, Y, inverted Y, U, and multi-branched forms (Table 2). At each site, I-shaped burrows were the most common. Multi-branched burrows were more frequent at St-Siméon than at Paspébiac, and U forms were absent in St-Siméon. The number of entire burrows per core ranged between 18 and 28.

Considering all depths and core replicates, the total number of burrow shafts visible on a 2-D section was greater in cores from St-Siméon (mean $\pm$ SE: 10.1 $\pm$ 1.2) than in those from Paspébiac (mean $\pm$ SE: 8.4 $\pm$ 1.3), but values were highly variable throughout (Figure 5A). At both sites, the number of shafts covaried with depth (Table 1), with the greatest number of shafts found close to the sediment surface. The relationship between burrow shafts and depth differed between sites: the slope was more pronounced for St-Siméon data, and the curves intersected at 70 mm depth (Figure 5A). The ANCOVA results showed significant intra-site variability but no site effect (Table 1).

The average diameter of shafts covaried with depth and varied significantly between cores (Table 1; Figure 5B). Average shaft diameter throughout the cores was 3.92 $\pm$ 0.07 mm at Paspébiac and 3.75 $\pm$ 0.61 mm at St-Siméon; the difference between sites was not significant (Table 1).

The percentage of space occupied by biogenic structures per CT section, when averaged throughout the length of the core, was greater and more variable at St-Siméon (mean $\pm$ SE: 9.84 $\pm$ 1.50%) than at Paspébiac (mean $\pm$ SE: 5.83 $\pm$ 0.33%). At St-Siméon, the percentage spatial occupation decreased with depth, whereas it appeared relatively constant with depth at Paspébiac (Figure 5C); the ANCOVA revealed a significant depth effect with a high variability among cores, but no significant difference between sites (Table 1).

Burrow volumes were highly variable and significantly different between sites (t-tests: TBV, $P < 0.05$; SLBV, $P < 0.05$). Average TBV was were slightly larger at St-Siméon (1957 $\pm$ 383 cm^3.m^{-2}) than at Paspébiac (1306 $\pm$ 151 cm^3.m^{-2}). Inter-site differences in SLBVs were more pronounced (St-Siméon: 1587 $\pm$ 457 cm^3.m^{-2}; Paspébiac 569 $\pm$ 300 cm^3.m^{-2}; Table 2).

The nearest-neighbour distance between burrow shafts ranged from 3.2 to 49.7 mm in the Paspébiac cores (mean $\pm$ SE: 19.06 $\pm$ 0.31 mm), and from 3.4 to 54.5 mm in the St-Siméon cores (mean $\pm$ SE: 20.06 $\pm$ 4.20 mm). The Clark & Evans index of aggregation is on average slightly lower at Paspébiac (mean $\pm$ SE: 1.06 $\pm$ 0.11) than at St-Siméon (mean $\pm$ SE: 1.36 $\pm$ 0.16) (Figure 6) with the difference between sites being nearly significant (Table 1). At Paspébiac, 11 out of 34 R measurements (32%) showed a significant departure from randomness at $P < 0.05$, compared to 23 out of 38 R measurements at St-Siméon (60%).

Relationships between variables

Several correlations between parameters were significant (Table 3), but coefficients were weak. TI values were highly negatively correlated to OM content and, to a lesser extent, with the percentage biogenic space and number of shafts. Also, the number of shafts and percentage biogenic space were positively correlated. When sites were considered separately, significant Pearson coefficients were greater between TI and OM in Paspébiac and between TI and number of shafts and percentage biogenic space in St-Siméon (Table 4).

DISCUSSION

Sediment-related characters at the study sites

As expected, sediment characteristics differed between study sites, likely as a result of different topographies and hydrodynamic conditions. Grain size, although determined at different depths (and lithofacies) in each core, showed certain site-specific trends. Grain size was more variable within the Paspébiac cores, and St-Siméon cores contained a greater proportion of mud and a higher organic matter content, suggesting that low energy conditions, vegetation and riverine input enhance sedimentation of finer particles at St-Siméon (Renaud, 2000). Site-specific differences in corer penetration (and resulting core length) were likely due to the relative position of a layer of dense, postglacial marine mud (Gagnoud et al., 2009), reflecting differences in the intensity and frequency of accretionary and erosionary (storm or ice-related) events in these intertidal areas. Dense postglacial mud limits the penetration of organisms of the *Macoma balthica* community such as *Alitta virens* (Gagnoud et al., 2009). It appears, therefore, that the relative thickness of habitable space in the upper sediment was greater at the Paspébiac site.

In CT-scanned sediment cores, the average TI usually increases with depth due to sediment compaction (Boespflug et al., 1995; Crémer et al., 2002; De Montety
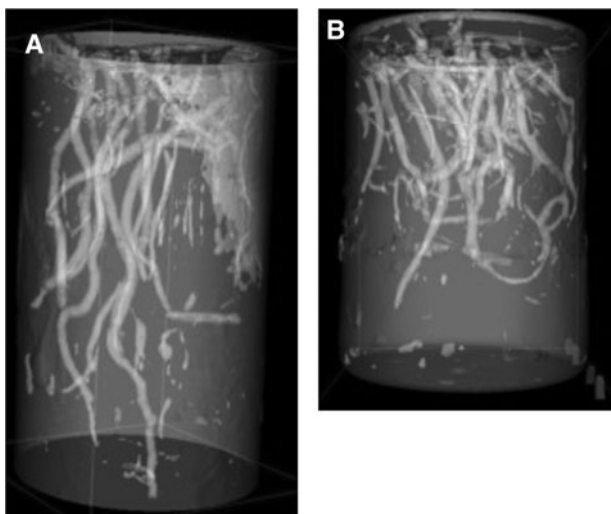

Fig. 4. Three-dimensional reconstruction of burrows in cores from (A) Paspébiac and (B) St-Siméon produced using OsiriX v.3.6.

Table 2. Number of burrows of each shape (I, Y, inverted Y, U and multi-branched) within the four sediment cores from each site, and calculated total biogenic volume (TBV) and surface-linked biogenic volume (SLBV).

	I	Y	Inverted Y	U	Multi-branched	Total number of burrows	TBV (cm³.m⁻²)	SLBV (cm³.m⁻²)
Paspébiac								
1	17	1	0	0	2	20	1012	47
2	19	1	3	0	2	25	1187	50
3	16	3	3	2	2	26	1724	1068
4	16	4	2	1	0	23	1301	1111
St-Siméon								
1	20	0	3	0	5	28	1830	1588
2	11	4	0	0	3	18	2162	2062
3	12	3	0	0	6	21	2355	1729
4	22	2	0	0	3	27	1484	970

et al., 2003; Dufour et al., 2005); the same trend was observed in all cores studied here. The average TI in a core section is affected by biogenic space, with core sections having a high proportion of (low TI) water-filled biogenic space having lower average TI values (Boespflug et al., 1995; Duliu, 1999; Pralle et al., 2001; De Montety et al., 2003). Boespflug et al. (1995) also found negative correlations between average TI values and both organic matter content and grain size. In our study, the average, depth-specific TI per section clearly differed in both study sites, and values are significantly, negatively correlated with organic matter content and to a weaker extent with the number of burrow shafts and the biogenic space occupied (Table 3). Average TI values of sediments from St-Siméon, the site with a greater proportion of mud and organic matter, were significantly lower than those from Paspébiac; this may be partly explained by differences in mineral composition between mud and sand (Crémer et al., 2002). Small-scale (intra-site) differences in TI values may be more reflective of the relative number and spatial coverage of biogenic structures, particularly at St-Siméon (Table 4).
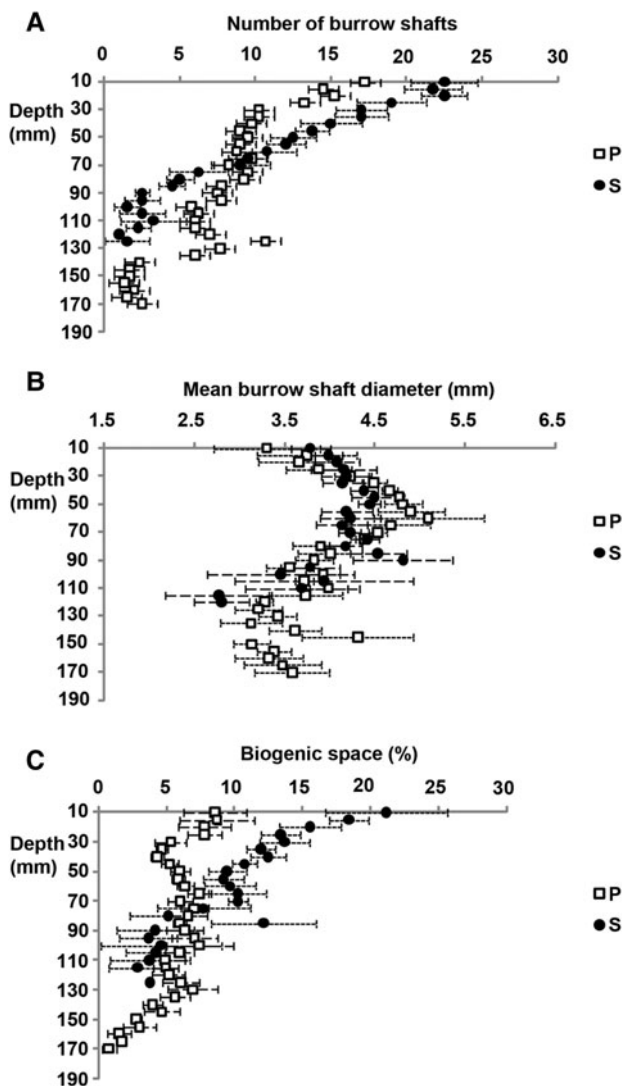
Burrow structure and distribution—general observations

Based on 3-D reconstructions, all burrow forms previously ascribed to A. virens (Miron et al., 1991b; Gingras et al., 2008; Herringshaw et al., 2010) were observed in the cores collected, with the exception of the L-forms of Miron et al. (1991b)—the latter may be artefacts of aquarium maintenance. At both sites, I-forms were most common. Different functions have been ascribed to those burrow forms: Y-shaped burrows may be associated with surface feeding activities and enhanced protection from predators
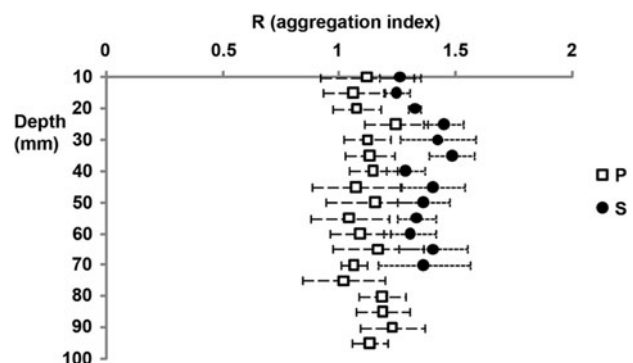

Fig. 5. Vertical distribution of (A) number of burrow shafts; (B) mean structure diameter; (C) percentage space occupied by structures, in cores sampled from Paspébiac (P) and St-Siméon. Values are averages ± SE of data from four cores per site.

Fig. 6. Aggregation index (R) determined for burrow shafts along a horizontal plane, according to depth in the core. Values are averages ± SE of data from four cores per site. P, Paspébiac; S, St-Siméon.

Table 3. Pearson correlations (R^2 values) between pairs of variables using all replicates of both sampling sites, combined. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

	Diameter	Number of shafts	% biogenic space	% organic matter	R
TI	−0.0819**	−0.1419***	−0.2659***	−0.7628***	−0.0094***
Diameter		−0.0009	−0.0008	0.0174	−0.0011
Number of shafts			0.4680***	0.0582	0.0604**
% biogenic space				0.1203*	0.0534*
% organic matter					0.0578

(Herringshaw *et al.*, 2010), while U-shaped burrows may be optimized for filter feeding (Gingras *et al.*, 2008). Form is also influenced by density: at low *A. virens* density (five individuals per m^{-2}), burrows are mostly U- or Y- shaped, while at high density (20 individuals per m^{-2}) I- and L-forms are dominant (Miron *et al.*, 1991b).

From the 2-D images obtained, we determined the number, diameter and space occupied by individual burrow shafts, as well as their spatial coordinates, at different depths within the cores. The total abundance and average diameter of burrow shafts were similar between sites. Overall, both the number of burrow shafts and percentage biogenic space decreased significantly with depth (Figure 5; Table 1), as observed previously (Mermillod-Blondin *et al.*, 2003; Michaud *et al.*, 2003; Rosenberg *et al.*, 2007, 2008); this is caused by both branching pattern (most forms have more shafts near the surface than at depth) and the presence of numerous short burrows. The average diameter of burrow shafts peaked at roughly 70 mm depth, likely due to the junction between branches in Y or multi-branched structures being larger in cross-section.

The 2-D images obtained by CT-scanning also allowed us to investigate the spatial distribution of burrow shafts at different depths within the sediment. At both sites, R values were relatively constant with depth (Figure 6), suggesting that the mechanisms leading to spatial patterning operate not only at the surface, but also within the sediment. Recent studies have documented pore-water pressure changes induced by the burrowing and irrigation activities of infaunal organisms; the sensing of characteristic pressure signals has been suggested as a potential mechanism for detecting competitors within the sediment (Wethey & Woodin, 2005). *Alitta virens* is sensitive to vibrations in water (Andzhon & Popov, 1979); similarly, pore-water pressure detection may drive spatial patterns within sediments.

The R index used here has certain weaknesses: the spatial scale chosen can influence the discrimination between random and aggregated distributions, and is more appropriate

when comparing large numbers of points (Andrew & Mapstone, 1987). Nonetheless, this index revealed differences in small-scale spatial patterns in burrow distributions between the study sites. The development of more complex indices for CT-scan, defining spatial distribution patterns in three dimensions, would be useful.

Burrow volume, both total (TBV) and considering only surface-linked structures (SLBV) could readily be determined from the CT-scanning data. However, determining SLBV was more challenging (and likely underestimated) because: (1) some burrows were seen to continue beyond the edge of the core, and therefore incomplete burrows were sampled (and likewise, some of the burrows assumed to be relict may have been be linked to the surface, beyond the core boundary); and (2) several burrows seemed to be obstructed near the sediment surface, possibly due to the activity of other bioturbating organisms, or related to sampling protocol. Nonetheless, biogenic volumes (or estimations thereof) can easily be obtained, and compared, using CT-scan data. Rosenberg *et al.* (2008) estimated a SLBV of 560 cm^3.m^{-2} for a subtidal benthic community dominated by *Amphiura filiformis* Müller 1776. This value is similar to the mean SLBV in Paspébiac and is one-third of the mean in St-Siméon (1729 cm^3.m^{-2}), reflecting how *A. virens* can greatly (and variably) impact and structure intertidal sediments.

Differences in *Alitta virens* burrows within and between sites

We had hypothesized that burrow characteristics would not differ significantly within a site. However, the number and diameter of shafts, percentage biogenic space, and R varied significantly among cores within a site, indicating significant patchiness at a decimetre to metre scale. For those same variables, ANCOVA results indicated that site effects were not significant; however, depth relationships of each of these variables, as seen in Figures 5 and 6, clearly show site-specific patterns (except for shaft diameter). Most likely, the ANCOVAs could not uncover inter-site differences because the curves intersected and crossed over at approximately 70 mm depth. Regardless (at least in the upper sediment column), burrow shafts were clearly more abundant and occupied more space at the more sheltered site, St-Siméon. We argue, therefore, that there were large-scale (site-specific) differences in most burrow characters, with the exception of shaft diameter. The relative thickness of habitable space, sediment characters, and possible differences in *Alitta virens* populations may have led to the observed differences.

The Clark & Evans R values indicated that burrow shafts were more randomly distributed at Paspébiac, and were more evenly distributed (i.e. maximally spaced) at

Table 4. Pearson correlations (R^2 values) between pairs of variables using all replicates within each sampling site, treated separately: Paspébiac (P) and St-Siméon (S). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

	Diameter	Number of shafts	% biogenic space	% organic matter	R
TI (P)	−0.0447*	−0.2787***	−0.1112***	−0.5954***	0.0161
TI (S)	−0.1636**	−0.6405***	−0.3889***	−0.0713	0.0094
% organic matter (P)	−0.0646	0.1936*	0.0315	–	−0.0338
% organic matter (S)	−0.0608	0.0031	0.0184	–	0.2293

St-Siméon, at all depths considered. The degree of spacing of burrow shafts within sediments may be influenced by how readily those sediments can transmit or propagate pore-water pressure signals. Additionally, the abundance of burrowing organisms on a small spatial scale (cm–dm) could impact distribution patterns through competitive or other interactions.

The discrepancy between TBV and SLBV was, in most cases, greater at Paspébiac (Table 2). The greater similarity between TBV and SLBV values in St-Siméon may be the result of greater sediment stability (and a better preservation of burrow orifices) at this site, possibly due to finer sediments increasing sediment cohesion. Alternatively, or additionally, a greater relative amount of SLBV in St-Siméon might be due to a greater number of burrow orifices at this site, as well as differences in burrow shape, size and distribution.

Usefulness of CT-scanning for studies of burrow structure

In this study, CT-scanning was successfully used to visualize the 3-D distribution of *A. virens* burrows and to study both sediment (i.e. TI) and burrow parameters in sediment cores. The TI of sediments was correlated with the number of burrow shafts, the biogenic space occupied, and sediment organic matter content. TI could, therefore, be a good descriptor of sediment characteristics of importance for biological processes. We also showed that CT-scanning is useful to investigate spatial patterns in biogenic spatial organization within sediments (in our case, using the R index), as well as to determine the biovolume and surface area occupied by biogenic structures. Beyond the usefulness of those measures in comparative studies, such metrics can (and should) be used to calibrate bioturbation models.

The small-scale variability observed here reinforces the need for replication in CT-scan studies, as suggested by Michaud *et al.* (2003). Ideally, larger cores should be collected for CT-scan analyses, particularly for studies of spatial patterns in relatively large organisms such as *Alitta virens*. Investigating further sites could also help us better understand the factors that drive spatial patterning in *A. virens*.

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