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Gigantism in Permian trepostomes from Greenland: testing the algal symbiosis hypothesis using $\delta^{13}C$ and $\delta^{18}O$ values

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ABSTRACT: Photosynthesizing endosymbiotic algae can result in gigantism in their hosts, but this has never been unequivocally documented in extant bryozoans. Unusually large colonies (up to 7 cm branch diameters) of the trepostome bryozoan *Tabulipora* sp. have been recovered from the Kungurian (Early Permian) Kim Fjelde Formation in eastern North Greenland. Håkansson & Madsen (1991) used carbon and oxygen isotope values from skeletal carbonate to test the hypothesis that the gigantism was caused by algal symbiosis. In this study, a more precise test of their hypothesis was conducted using a finer sampling protocol on a colony from the same formation and location. Skeletal carbonate reveals mean $\delta^{13}C$ and $\delta^{18}O$ values of 3.9 ‰ VPDB and -6.5 ‰ VPDB, respectively. Diagenetic effects were evaluated by discretely recovering cements contained within zooecial chambers; skeletal values are significantly higher than the surrounding cements. In consideration of the isotope value of the Permian ocean, it is concluded that this isotopic evidence is largely negative. We reject the algal symbiosis hypothesis based on the combined isotopic, morphologic, and paleoenvironmental evidence.

1 INTRODUCTION

Gigantism refers to a condition in organisms with a larger body size than in phylogenetically related organisms. It can be caused by disease (e.g. pathologic gigantism) or special growth conditions that result in rapid growth and/or longevity (e.g. island gigantism, polar gigantism, abyssal gigantism, or symbiotic gigantism). In particular, elevated dissolved oxygen levels are most commonly cited as the source of gigantism due to special growth conditions (Chapelle and Peck 1999). Symbiotic gigantism results from the presence of a wide range of photosynthesizing endosymbiotic organisms, but most common are the 'zooxanthellae' algae which are single-celled autotrophic plants (dinoflagellates) that live symbiotically in the tissues of their heterotrophic animal hosts (Trench 1993). Symbiotic algae photosynthesize and release photosynthetic products (sugars and O_2), into the tissues of their host. The host metabolizes the photosynthetic products and releases CO_2 and waste. The symbiotic algae absorb the CO_2 and extract the nutrients from the waste (phosphate and nitrate) during photosynthesis. This allows organisms to live in nutrient poor environments (Stanley 2003). Additionally, the symbiotic algae receive protection from predators by living in their host's tissues. This symbiosis with its efficient recycling of nutrients promotes calcification (Pearse and Muscatine 1971) sometimes at 'staggering' rates which may lead to skeletal gigantism (Hallock 1981, 1996, Cowen 1983). This is primarily achieved in two ways: removal of CO_2 from the host's tissues helps convert bicarbonate to carbonate and the addition of energy allows for calorically expensive calcification. Algae-bearing hosts are generally an order of magnitude larger than their non-symbiont-bearing relatives (Cowen 1983). Algal symbiosis has resulted in gigantism in a wide variety of clades, most notably foraminiferans (Lee & Anderson 1991), corals (Coates & Jackson 1987), and bivalves (Morton 2000). These extant clades also have extinct ancestors in the fossil record whose gigantism has also been attributed to algal symbiosis (e.g. foraminiferans (Lee et al. 1979), corals (Cowen 1988), and bivalves (Vogel 1975)).

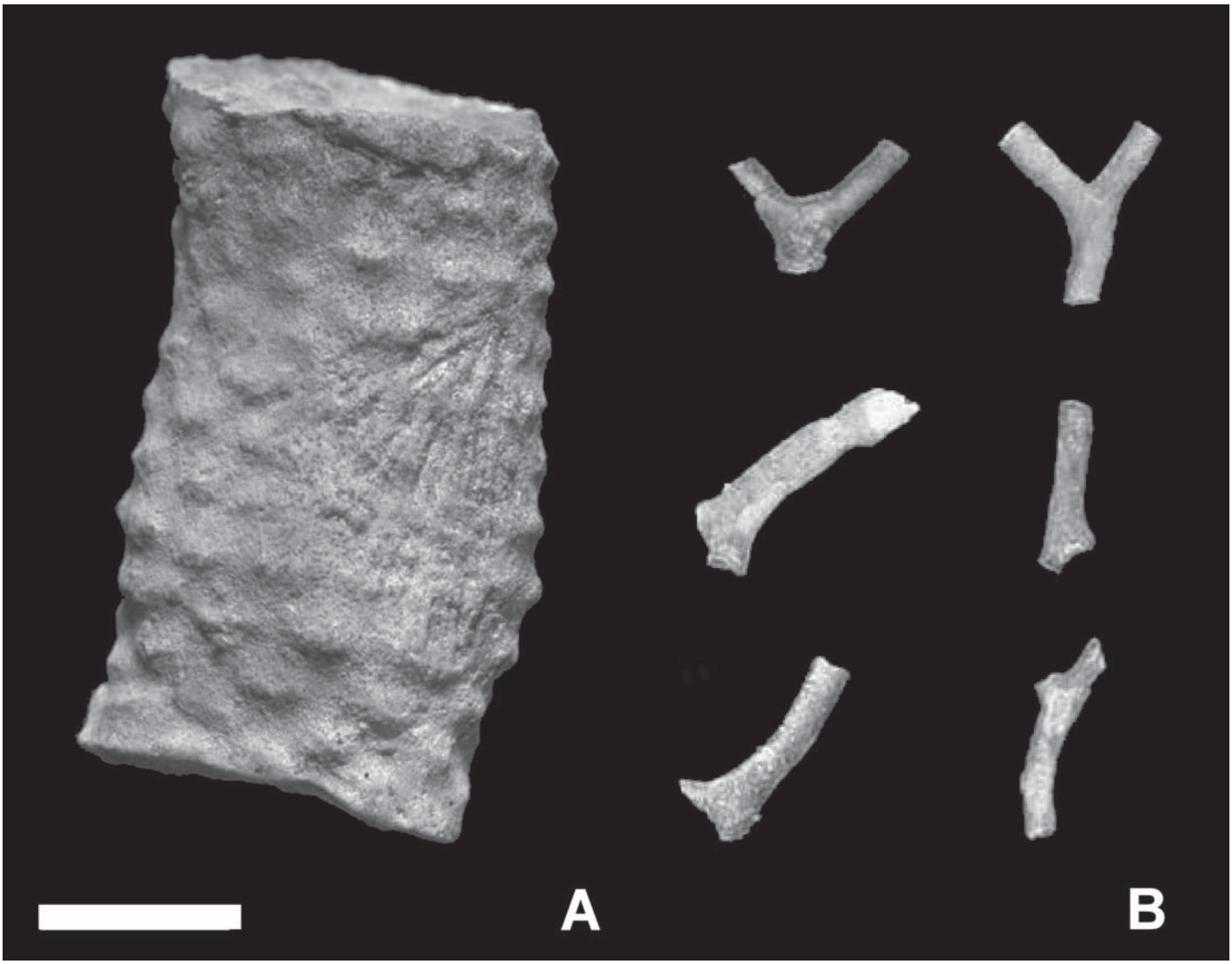

Figure 1. Colony branch fragment of the 'gigantic' Permian bryozoan *Tabulipora* sp. (GI 90635-1) (A) is 63 mm long and 39 mm wide compared to six 'regular sized' *Tabulipora* sp. branch fragments from the Pennsylvanian of Kansas (B). Scale bar = 20 mm.

1.1 *Gigantism in bryozoans*

Gigantism has been recognized in various bryozoans, including several Ordovician trepostomes (Raizen et al. 1999), the Mississippian trepostome *Stenophragmidium* sp. with >35 mm diameter branches (Wyse Jackson & Kora, unpublished data), the Cretaceous cyclostome *Pennipora anomalopora* with 22 mm diameter branches (Taylor & Voigt 1999), the Pleistocene cheilostome *Schizoporella* sp. (Cuffey & Fonda 1976), and the Recent cheilostome *Cellepora coronopus* with 20 mm diameter branches (Chapman 1933). Larger than all these is the Permian trepostome *Tabulipora* sp. from the Kim Fjelde Formation in Greenland (Fig. 1) in which branches reached up to 70 mm in diameter and 200 mm in length (Ross & Ross 1962, Håkansson 1979, Madsen 1987, 1994, Madsen & Håkansson 1989, Håkansson & Madsen 1991, Stemmerik 1997). These Greenland colonies are at least an order of magnitude larger than other stenolaemates specifically and bryozoans in general (Madsen 1991). In addition, Håkansson & Madsen (1991) argued that this example of gigantism was the result of endosymbiotic algae.

1.2 *History of research on endosymbionts in bryozoans*

Previously in earlier bryozoan research, plant cells were reported in brown bodies or in the gut wall of bryozoans (MacMunn 1887, Oltmans 1923, Zirpolo 1923). The cells in brown bodies most likely represent undigested food (Schopf 1977). Reports of cells in the gut wall are of more interest to this study. Of these previous studies, Zirpolo's (1923) is most unequivocal in attributing the cells to symbiotic algae in the gut wall, but Buchner (1930) was unable to substantiate Zirpolo's claim. Bryozoan-algal relationships were again invoked to explain zoarial habits of some Paleozoic fossil species including *Archimedes* sp. (Condra & Elias 1944, 1945). This bryozoan-algal relationship was quickly disproved (Easton 1944, Haas 1945, Shulga-Nesterenko 1949), but even if these relationships were genuine, they involved exosymbiotic, multicellular algae, not the endosymbiotic, unicellular algae that are the focus of this paper. Lutaud (1965) showed that bacterial cysts live within bryozoans, but once again they were non-symbiotic. This was the state of bryozoan-algae symbiosis until

Cuffey (1970, p. 44) wrote, 'Single-celled algae (zooxanthellae) live commensally in the soft tissues of a few modern marine bryozoans.' But this statement was unsupported and was thus rejected by Schopf (1977, p. 181) who found no evidence that any modern bryozoan has a symbiotic relationship with single-celled algae. Taylor (1999) reviewed the literature and concluded that there are no known examples of modern bryozoans with skeletons which are modified by the presence of photosynthetic or chemosynthetic endosymbionts. Since then, Crowley & Taylor (2000) reported on hydroids living symbiotically with some ascophoran cheilostomes, and Kaselowsky et al. (2002) documented the presence of fungi growing in the metacoelom of bryozoans causing the formation of 'giant buds'. These are not the photosynthesizing endosymbiotic algae common in reef forming corals.

The most rigorous test to date for symbiotic algae in bryozoans came from a study by Håkansson & Madsen (1991) who argued that for some Permian bryozoans gigantism was the result of endosymbiosis. The effectiveness of Håkansson & Madsen's original isotopic test of the algal symbiosis hypothesis for gigantism in bryozoans was limited by the sampling resolution of the technology available at the time. New micromilling technology with 1 m spatial sampling resolution and new mass spectrometers that require ~20 μg samples (sensu Wurster et al. 1999) allow better independent sampling of bryozoan skeletal walls and secondary cements. This is in contrast to Håkansson & Madsen's (1991) ≥ 1.5 mg samples. They recognized this on p. 154: 'due to the comparatively high amount of carbonate material required by the available analytical facilities, all bryozoan samples analyzed contain a varying proportion of the diagenetic cement spar now occupying all zooidal cavities.' It is the goal of this study to test the algal symbiosis hypothesis for gigantism in the Permian trepostome *Tabulipora* sp. using state-of-the-art stable isotope analytical technology.

2 MATERIALS

This study was based on a single Geological Institute of Copenhagen specimen (GI 90635-1) of the stenoporid trepostome bryozoan *Tabulipora* sp. The colony branch has a length of 63.0 mm and a diameter of 38.9 mm (endozone diameter = 20.7 mm, exozone width = 9.1 mm). The sample was collected 10 km northeast of Kap Jungersen in southern Amdrup Land in eastern North Greenland (Håkansson et al. 1981, Fig. 17, un-numbered dot NE of locality 16; (80°35′N, 15º59′W). It came from the top of the Kim Fjelde Formation of the Mallemuk Mountain Group (Håkansson 1979, Stemmerik & Håkansson 1989) and has an age of Early Permian (Kungurian stage) (Stemmerik et al. 1998). The Kim Fjelde Formation was deposited in the Wandel Sea Basin which developed in response to extension and rifting between Greenland, Norway and Spitsbergen (Håkansson & Stemmerik 1989, Stemmerik et al. 1998). Deposition in the basin was controlled by a series of syndepositional extensional faults and grabens that developed parallel to the stable Greenland craton (Stemmerik et al. 1996). Sedimentation in Amdrup Land was restricted to a subsiding platform bordered to the west by the East Greenland fault zone and to the northeast by the Sommerterasserne fault (Håkansson & Stemmerik 1995).

This formation consists of cliff-forming, normal marine, shallow to deeper shelf, finely bedded, chert-rich, fossiliferous limestones (Håkansson 1979, Håkansson et al. 1981, Stemmerik & Håkansson 1989, Stemmerik et al. 1996). The giant bryozoans are preserved in a bryozoan rudstone facies which was deposited on the outer shelf below storm wave base (facies F4 of Stemmerik 1997). Stemmerik (1997) correlated this facies with modern, shelf edge, cool-water carbonates deposited in 140-250 m depth (James et al. 1992). This interpretation is supported by the presence of contemporaneous formations at slightly lower paleolatitude in the Norwegian-Greenland Sea Basin of East Greenland which reflect warmer water conditions (Stemmerik 1995) and at slightly higher paleolatitude in the Sverdrup Basin of Canada which reflect even cooler water conditions (Beauchamp & Desrochers 1997). The cold temperate conditions are also reflected in the diagenetic products in the Kim Fjelde Formation where LMC cements dominate (Håkansson & Stemmerik 1995).

The general Late Paleozoic northward movement of Pangea and the cessation of warm water currents in response to mid-Permian, Proto-Atlantic rifting led to a shift to temperate cool-water carbonates in eastern North Greenland during the Kungurian (Beauchamp 1994, Stemmerik & Worsley 1995). This cooling has been linked to changes in the composition of the Permian bryozoan faunas of the higher latitudes in the northern hemisphere (Ross 1995). In eastern North Greenland, sedimentation occurred in cold temperate water (Stemmerik et al. 1996). The Kim Fjelde Formation was deposited in the Early Permian pan-Arctic shallow carbonate platform (Håkansson & Stemmerik 1984, 1989, Stemmerik & Håkansson 1989) from 35°N (Scotese & Langford 1995) or 40°N paleolatitude (Coward 1995, Ziegler et al. 1997).

3 METHODS

The bryozoan colony was mounted on a glass slide and thick sectioned (100 μm thick), and its exposed

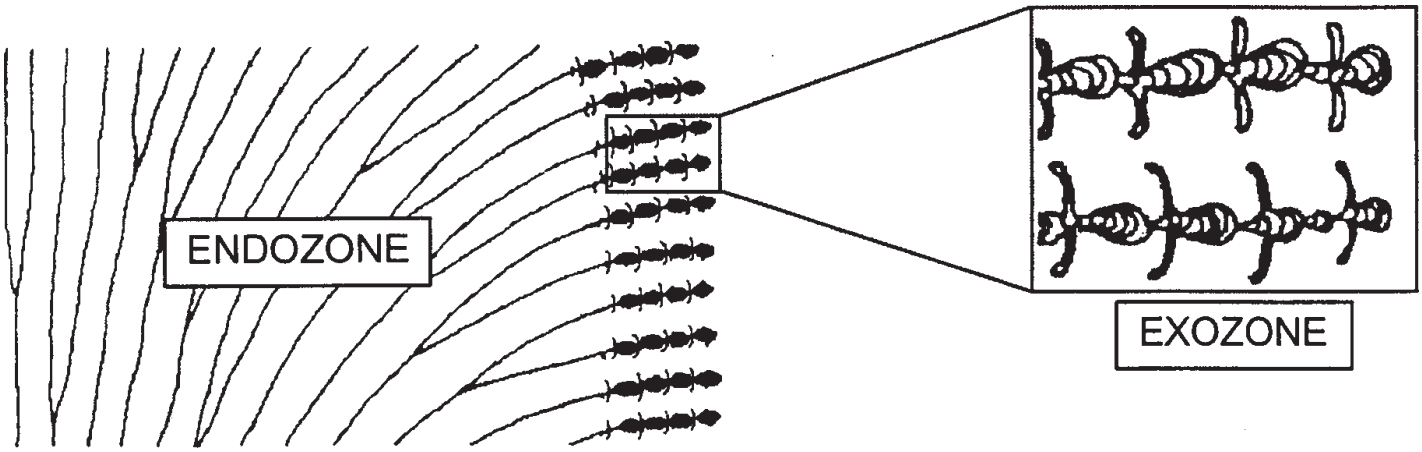

Figure 2. Diagrammatic longitudinal cross section view of *Tabulipora* sp. showing the endozone with its thin (~10 μm) zooecial skeletal walls in contrast with the exozone with its thick (~150 μm) zooecial skeletal walls.

upper surface was polished. Micromilling was performed (sensu Wurster et al. 1999) on a robotic computer controlled three-dimensional positioning stage set under a fixed high-precision dental drill that results in 1 μm spatial sampling resolution. Enough carbonate was milled to generate samples of approximately 20 μg of powder for each carbon and oxygen isotope analysis. The carbonate samples were roasted *in vacuo* at 200°C to remove water and volatile organic contaminants that could interfere with carbonate analyses. Stable isotope values were obtained using a Finnigan Kiel-III automated carbonate preparation system directly coupled to the inlet of a Finnigan MAT 252 gas ratio mass spectrometer. Carbonate was reacted at 70°C with two drops of anhydrous phosphoric acid for 90 seconds. Isotope ratios were corrected for acid fractionation and ^{17}O contribution and reported in per mil notation relative to the VPDB standard. Precision and calibration of data were monitored through daily analysis of NBS-18 and NBS-19 carbonate standards. $\delta^{18}O$ values of the samples are bracketed by those of the standards. Precision is better than ± 0.1 for both carbon and oxygen isotope values. All isotope values in this paper are presented as $\delta^{18}O_{(CaCO3)}$ and $\delta^{13}C_{(CaCO3)}$ relative to PDB.

The endozone and exozone were sampled separately as were the diagenetic infilled cements and the zooecial skeletal walls (Figs 2-3). The endozone is thin-walled (~10 μm) so relatively more diagenetic cement was probably included in the carbonate samples. The exozone is thick-walled (over 150 μm) so relatively less diagenetic cement was probably included in the carbonate samples.

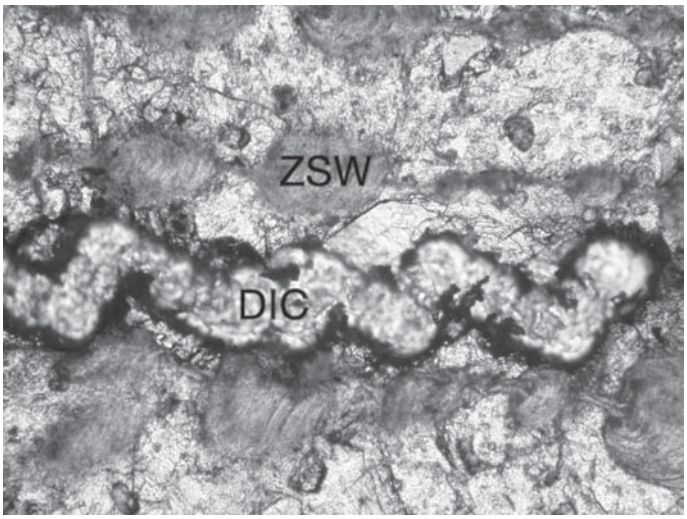

Figure 3. Photomicrograph of the exozone of *Tabulipora* sp. (GI 90635-1) showing examples of separate sampling areas for zooecial skeletal walls (ZSW) and diagenetic infilled cements (DIC). Image is 1 mm wide.

4 ISOTOPIC EFFECTS OF PHOTOSYNTHESIZING ENDOSYMBIONTS

Animals that do not secrete shell material in isotopic equilibrium with the surrounding water possess a vital effect (Lowenstam & Epstein 1954). The effect of photosynthesizing endosymbionts on stable isotope values of host organisms' skeletons is complex and equivocal (Houston et al. 1999). Carbon isotope fractionation is complex as it is affected by a variety of factors (i.e. vital effects) including kinetic effects, that are controlled by growth and calcification rates, as well as metabolic effects, that are controlled by respiration and photosynethetic rates (Norris 1998). In contrast, $\delta^{18}O$ fractionation is relatively simple as it is predominantly correlated with water temperature and salinity (Norris 1998).

Conventional wisdom now is that the vital effect of symbionts is expressed as higher skeletal $\delta^{13}C$ values (e.g. Barrera et al. 1990, Stanley & Swart 1995, Norris 1996, 1998). This is in contrast to earlier studies that found the opposite (e.g. Weber & Woodhead 1970, Erez 1978, McConnaughey 1989). Conventional wisdom now also holds that the vital effect of symbionts is expressed as lower $\delta^{18}O$ values (e.g. Weber & Woodhead 1970, 1972, Buchardt & Hansen 1977, Erez 1978,

Table 1. Summary of stable isotopic values from the Permian bryozoan *Tabulipora* sp. (GI 90635-1). n = number of samples.

General sample location	Specific sample location	n	$\delta^{13}C$ (‰ VPDB) Range	Mean	Standard deviation	$\delta^{18}O$ (‰ VPDB) Range	Mean	Standard deviation
Endozone		11	3.4 to 4.1	3.7	0.18	-8.1 to -5.4	-7.3	0.77
	Diagenetic infilled cements	7	3.4 to 4.1	3.7	0.23	-8.1 to -5.4	-7.4	0.95
	Zooecial skeletal walls	4	3.7 to 3.8	3.8	0.03	-7.2 to -6.9	-7.1	0.18
Exozone		9	3.4 to 4.1	3.7	0.24	-8.2 to -5.5	-7.1	1.22
	Diagenetic infilled cements	5	3.4 to 3.7	3.6	0.09	-8.2 to -7.1	-7.9	0.44
	Zooecial skeletal walls	4	3.7 to 4.1	4.0	0.18	-7.5 to -5.5	-6.0	1.01

McConnaughey 1989, Norris 1996, Houston et al. 1999), while other studies have found that symbionts have no effect (e.g. Barrera et al. 1990, Crowley & Taylor 2000). Similarly, the conventional wisdom is that $\delta^{13}C$ and $\delta^{18}O$ are positively correlated only in organisms lacking photosynthesizing endosymbionts (e.g. McConnaughey 1989, Stanley & Swart 1995, Spero et al. 1997), although others have suggested that symbiotic organisms can exhibit a similar relationship (e.g. Weber & Woodhead 1970, Romanek & Grossman 1989). To further complicate matters, some workers argue the positive correlation is in response to kinetic fractionation, not simply the metabolic effect of symbionts (e.g. McConnaughey 1989, Norris 1998, Crowley & Taylor 2000). Thus, using stable isotope values to differentiate between symbiotic and asymbiotic taxa in the fossil record is not at all straight forward. For the purposes of this study, it is assumed that the presence of photosynthesizing endosymbionts will be expressed as higher $\delta^{13}C$ and lower $\delta^{18}O$ values, with no positive correlation between the two.

5 RESULTS

Twenty carbonate samples were collected for stable isotope analysis (Table 1, Fig. 4). For the colony as a whole, the zooecial skeletal walls (mean $\delta^{13}C$ = 3.9 ‰ VPDB, mean $\delta^{18}O$ = -6.5 ‰ VPDB) displayed significantly higher values than the diagenetic infilled cements (mean $\delta^{13}C$ = 3.6 ‰ VPDB, mean $\delta^{18}O$ = -7.6 ‰ VPDB)(t-Tests, $P < 0.019$). For the exozone in particular, zooecial skeletal walls also had significantly higher $\delta^{13}C$ and $\delta^{18}O$ values than diagenetic infilled cements (t-Tests, $P < 0.025$). In contrast, endozonal cement and skeletal wall samples were not significantly different in either $\delta^{13}C$ or $\delta^{18}O$ mean values (t-Tests, $P > 0.05$). This probably reflects mixing of the zooecial skeletal wall carbonate and the diagenetic infilled cement carbonate in the thin-walled endozone. This could happen if the targeted cement was relatively thin and the drill penetrated the underlying zooecial wall. This mixing can be seen in Figure 4 where the arrow in the upper right corner may reflect inclusion of some skeletal carbonate in the cement.

6 DISCUSSION

In order to interpret these results, the original isotope values of the contemporaneous seawater must be known. The global mean isotope value of Early Permian (Kungurian stage) seas has been inferred from various sources such as LMC brachiopod skeletons. In the Early Permian $\delta^{13}C$ values are consistently estimated at between +4 and +6 ‰ VPDB, and $\delta^{18}O$ values are estimated at between -4 and -6 ‰ VSMOW (Gruszczynski et al. 1989, Scholle 1995, Mii et al. 1997, Veizer, et al. 1999). Whole rock analyses from the same general age and location as this study yield similar $\delta^{13}C$ values, but a wider range of $\delta^{18}O$ values: between -3 and -9 ‰ (Stemmerik & Magaritz 1989). Lower ^{18}O values are interpreted as diagenetic overprinting (Stemmerik & Magaritz 1989). Using an intermediate value for the mean isotope values of the Early Permian seawater of $\delta^{13}C$ = +5 ‰ VPDB and $\delta^{18}O$ = -5 ‰ VSMOW (Gruszczynski et al. 1989, Scholle 1995, Mii et al. 1997, Veizer, et al. 1999), our least diagenetically altered samples (i.e. exozone skeletal walls) display $\delta^{13}C$ values and $\delta^{18}O$ values that are 1.0 ‰ lower (i.e. 5.0 - 4.0 ‰ and -5.0 - -6.0 ‰, respectively). $\delta^{13}C$ and $\delta^{18}O$ values are significantly, positively correlated ($m = 0.20$, $R^2 = 0.88$, $P < 0.01$; Fig. 4).

Isotope values do not reveal an obvious signal of photosynethsizing endosymbionts. The lack of higher $\delta^{13}C$ and a positive correlation between $\delta^{13}C$ and $\delta^{18}O$ values argue for their absence. The only isotopic evidence for their presence are lower $\delta^{18}O$ values, but these could simply represent a minor diagenetic signal.

Our data agree well with those of Håkansson & Madsen (1991) giving confidence to the analyses made many years apart in different laboratories (Fig. 5). Their mean $\delta^{13}C$ value was 3.6 ‰ compared to our mean of 4.0 ‰ VPDB, and their mean $\delta^{18}O$ value was -6.4 ‰ VPDB compared to our mean of -6.0 ‰

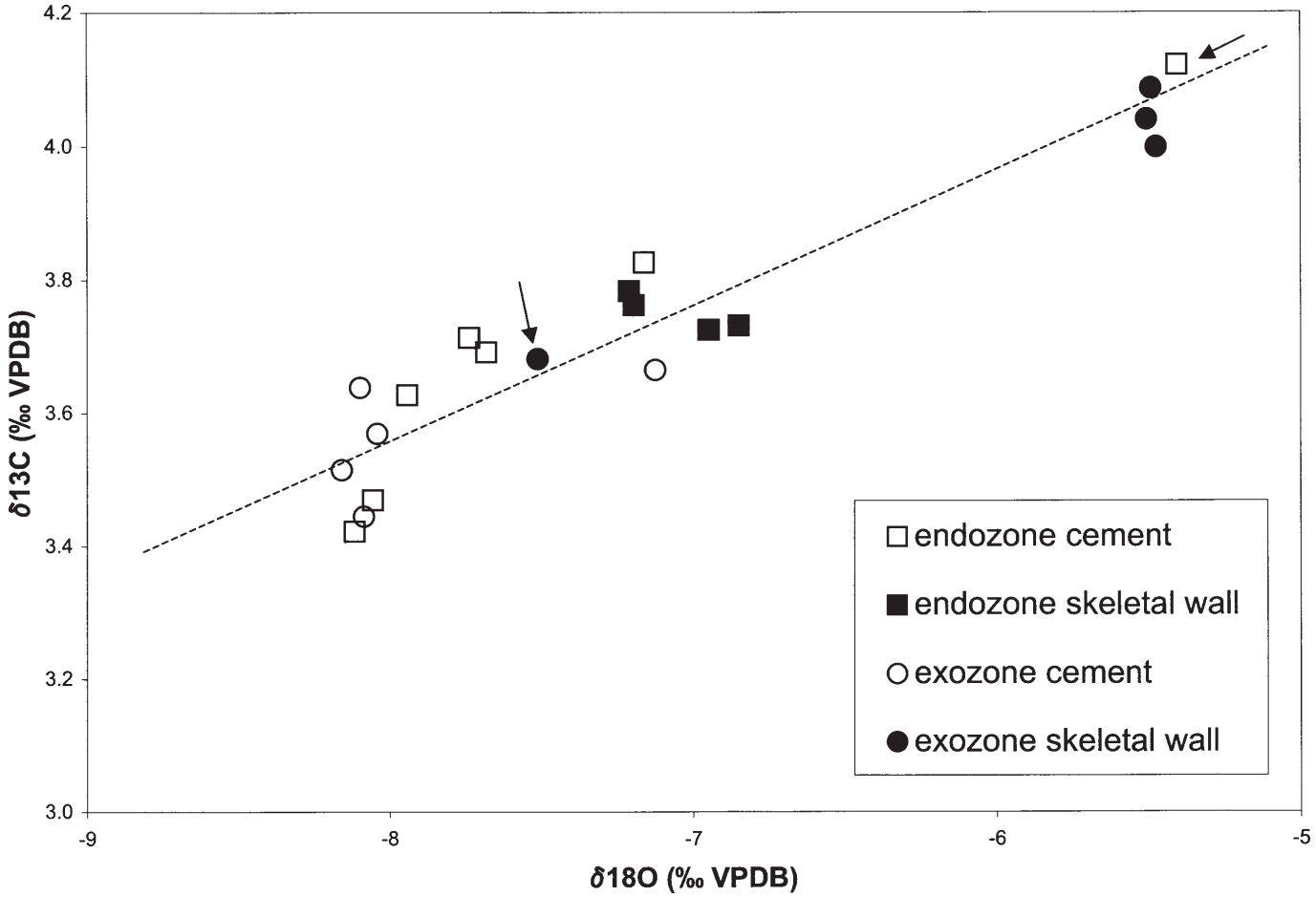

Figure 4. Plot of $\delta^{13}C$ and $\delta^{18}O$ values from colony of *Tabulipora* sp. (GI 90635-1). Dashed line represents linear regression (m = 0.20, $R^2 = 0.89$, $P < 0.01$). Right arrow indicates possible contamination of cement sample by skeletal wall carbonate. Left arrow indicates possible contamination of skeletal wall sample by cement.

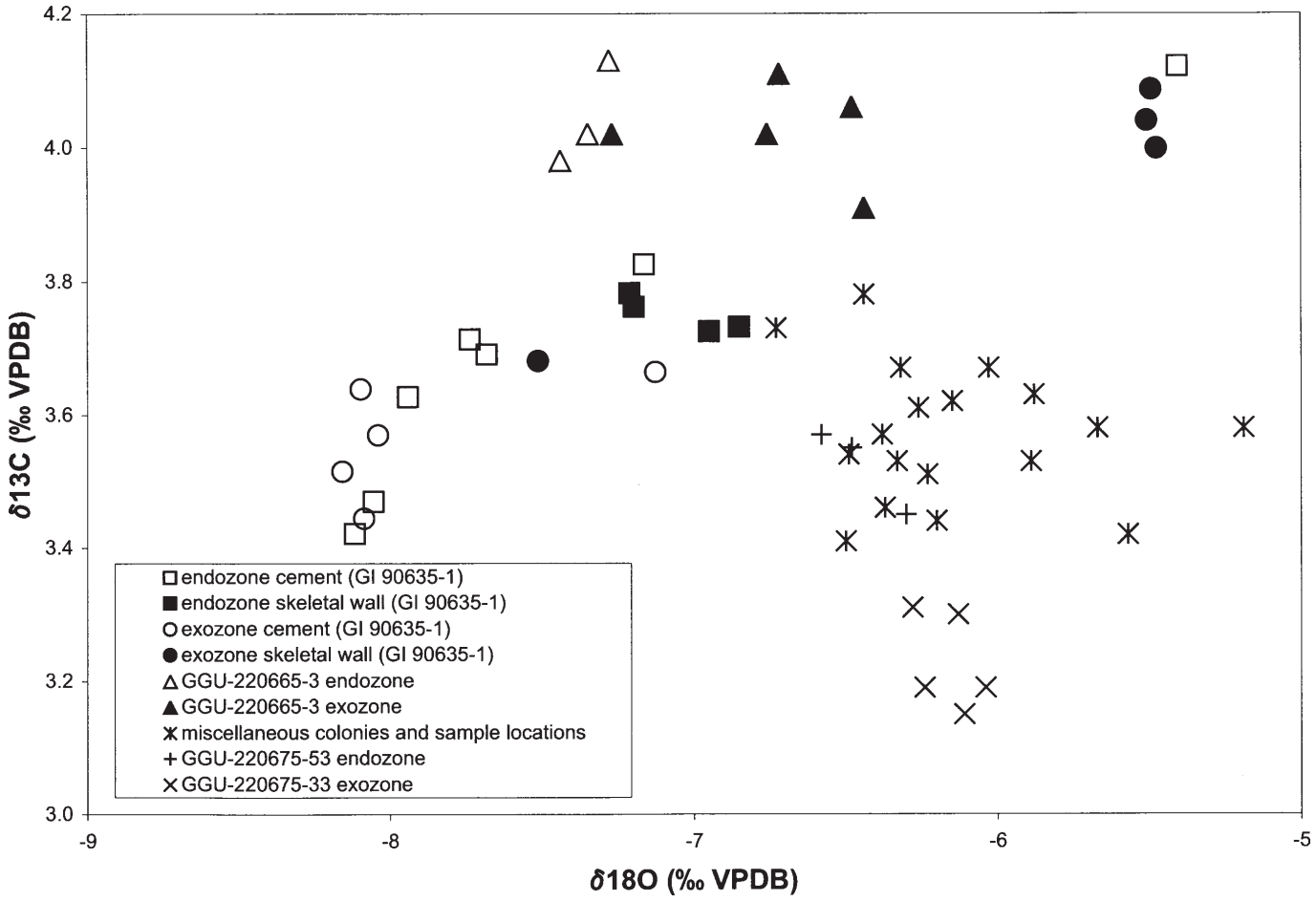

Figure 5. Plot of $\delta^{13}C$ and $\delta^{18}O$ values from colony of *Tabulipora* sp. from this study (GI 90635-1) compared to original data from Håkansson & Madsen (1991) (GGU-220665-3, GGU-220675-53, and miscellaneous colonies).

Table 2. List of 17 characteristics that can be used to infer the presence of photosynthesizing endosymbiotic algae in fossil hosts and how each was scored for the bryozoans in this study. Modified from Cowen (1983) and Stanley & Swart (1995).

Indirect evidence of photosynthesizing endosymbiotic algae in host	Is this true for the bryozoan in this study?	Qualifications
- Large size	Yes	See Figure 1.
- Rapid growth	?	No evidence.
- Restricted to simple phyla	No	But other hosts with photosynthesizing endosymbiotic algae (e.g. bivalves, nudibranchs, and ascidians and possibly even brachiopods as well) are not simple.
- Most commonly are filter feeders	Yes	But foraminiferans and nudibranchs with photosynthesizing endosymbiotic algae are exceptions.
- High surface area-to-volume ratios	Yes	But the robust branch sizes reduce the ratios compared to other less robust ramose species. But bivalves with photosynthesizing endosymbiotic algae fail this test as well.
- High skeleton-to-body ratios	Yes	But flatworms and nudibranchs with photosynthesizing endosymbiotic algae are exceptions.
- Soft tissue layers on the photic side	Yes	But the undersides of branches would have been shaded, but bivalves with photosynthesizing endosymbiotic algae partly fail this test as well.
- Thin tissue layers for light to pass through	Yes	In general, the confluent outer membrane of trepostomes is thin. Specifically, *Tabulipora*'s growing tip has been interpreted as a greenhouse (Håkansson & Madsen 1991, Text-Fig. 3)
- Grow toward light	Yes	But the ramose growth habit could also be in response to growth toward plankton which is the same general direction as light.
- High level of colonial integration	Yes	This species has giant macular feeding structures (Key et al. 2002).
- Most common in oligotrophic reef environments	No	The closest contemporaneous reefs are found further south in the Norwegian-Greenland Sea Basin of East Greenland (Stemmerik 1995).
- Restricted to shallow water in photic zone	No	Stemmerik (1997) correlated this facies with modern, shelf edge, cool-water carbonates deposited in 140-250 m depth.
- Restricted to low turbidity water	?	No evidence.
- Restricted to tropics with minimal fluctuations in seasonal light intensity	No	35-40°N paleolatitude.
- Higher $\delta^{13}C$	No	But see Discussion.
- Lower $\delta^{18}O$	Yes	But see Discussion as this could be due to diagenesis.
- Lack of positive correlation between $\delta^{13}C$ and $\delta^{18}O$ values	No	But see Discussion.

VPDB. Our interpretations based on the newest literature reviewed in section 4 differ as Håkansson & Madsen (1991) interpreted lower $\delta^{13}C$ values as indicative of photosynthesizing endosymbionts.

Due to the lack of direct evidence of photosynthesizing endosymbiotic algae in the fossil record (i.e. discovery of the endosymbionts themselves), indirect evidence such as isotopes must be used. Are there other independent characters that can be used to test the algal symbiosis hypothesis? Based on Cowen (1983) and Stanley & Swart (1995), 17 characteristics of hosts with photosynthesizing endosymbiotic algae were identified (Table 1). Some were morphological, others were environmental, while others were geochemical. The Permian *Tabulipora* sp. from Greenland was examined for each of these (Table 1).

7 CONCLUSIONS

Weighing equally each of the 17 characteristics of indirect evidence for the presence of photosynthesizing endosymbiotic algae in the host bryozoans, the count is nine in support, six in opposition, and two unresolved (Table 2). Of the nine supporting characteristics, six are qualified with some doubt leaving three definite lines of supporting evidence. These are large size (the original impetus for the paper), thin tissue layers for light to pass through, and a high level of colonial integration. Of the six in opposition, three are qualified with some doubt leaving three definite pieces of contradictory evidence. These are most common in oligotrophic reef environments, restricted to shallow water in photic zone, and restricted to tropics with minimal fluctuations in seasonal light intensity. The morphological evidence was largely in favor of endosymbiosis (8 in support, 1 in opposition, and 1

equivocal). The paleoenvironmental evidence was largely against endosymbiosis (0 in support, 3 in opposition, and 1 equivocal). The isotopic evidence was also largely against endosymbiosis (1 in support, 2 in opposition, and 0 equivocal). Thus the evidence for the presence of photosynthesizing endosymbiotic algae in these bryozoans is at best equivocal.

In the absence of direct evidence for endosymbionts, Cowen (1983) reasoned for the most conservative approach to testing this hypothesis in fossils by arguing that any single piece of negative evidence requires the rejection of the algal symbiosis hypothesis. Based on the presence of three qualified negative pieces of evidence and three unqualified negative pieces of evidence, the algal symbiosis hypothesis for gigantism in these bryozoans is rejected.

The gigantism in *Tabulipora* sp. may have simply been a function of exposure to ideal growing conditions. Gigantism has been long known from other Permian faunas (e.g. Hayasaka & Hayasaka 1953), so perhaps it was simply an environmental effect. This is supported by the fact that these bryozoans are not the only large elements of their fauna, as there are also large productid and spiriferid brachiopods (Håkansson & Stemmerik 1995) and giant colonies of the bryozoan *Amphiporella* sp. (Madsen 1994, Stemmerik 1997).

This lack of an endosymbiotic vital effect is good news for those using bryozoan skeletons for stable isotope-based temperature calculations. It is generally assumed that bryozoan skeletons are secreted in isotopic equilibrium with their surrounding water. The data on which this is based is limited but growing and indicates the majority of bryozoans secrete their skeletons in isotopic equilibrium (Forester et al. 1973, Pätzold et al. 1987, Wefer & Berger 1991, Rao & Nelson 1992, Rao 1993, Bone & James 1997, Rahimpour-Bonab et al. 1997, Crowley & Taylor 2000, Machiyama et al. 2002, Smith & Key 2004, Smith et al. in press). The most notable exceptions are documented in Crowley & Taylor (2000) and Smith et al. (in press).

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REFERENCES

Barrera, E., Tevesz, M.J.S. & Carter, J.G. 1990. Variations in oxygen and carbon isotopic compositions and microstructure of the shell of *Adamussiun colbecki* (Bivalvia). *Palaios* 5: 149-159.

Beauchamp, B. 1994. Permian climatic cooling in the Canadian Arctic. In G.D. Klein (ed.), *Pangea: paleoclimate, tectonics and sedimentology during accretion, zenith and breakup of a supercontinent*: 229-245. Boulder: Geological Society of American Special Paper 228.

Beauchamp, B. & Desrochers, A. 1997. Permian warm- to very cold-water carbonates and cherts in northwest Pangea. In N.P. James & J.A.D. Clarke (eds), *Cool-water carbonates*: 327-347. Tulsa: SEPM Special Publication No. 55.

Bone, Y. & James, N.P. 1997. Bryozoan stable isotope survey from the cool-water Lacepede Shelf, southern Australia. In N.P. James & J.A.D. Clarke (eds), *Cool-water carbonates*: 93-105. Tulsa: SEPM Special Publication No. 55.

Buchardt, B. & Hansen, H.J. 1977. Oxygen isotope fractionation and algal symbiosis in benthic foraminifera from the Gulf of Elat, Israel. *Bulletin of the Geological Society of Denmark* 26: 185-194.

Buchner, P. 1930. *Tiere und Pflanze in Symbiose*. Berlin: Borntraeger.

Chapelle, G. & L.S. Peck. 1999. Polar gigantism and oxygen. *Nature* 399: 114-115.

Chapman, F. 1933. On a gigantic polyzoan referable to *Lichenopora*, from the Miocene of Airey's Inlet, Victoria. *Proceedings of the Royal Society of Victoria* 46: 55-59.

Coates, A.G. & Jackson, J.B.C. 1987. Clonal growth, algal symbiosis, and reef formation by corals. *Paleobiology* 13: 363-378.

Condra, G.E. & Elias, M.K. 1944. Study and revision of *Archimedes* (Hall). *Geological Society of America Special Paper* 53: 1-243.

Condra, G.E. & Elias, M.K. 1945. *Bicorbula*, a new Permian Bryozoan, probably a bryozoan-algal consortium. *Journal of Paleontology* 19: 116-125.

Coward, M.P. 1995. Structural and tectonic setting of the Permo-Triassic basins of northwest Europe. In S.A.R. Boldy (ed.), *Permian and Triassic Rifting in Northwest Europe*: 7-39. Geological Society Special Publication. No. 91.

Cowen, R. 1983. Algal symbiosis and its recognition in the fossil record. In M.J.S. Tevesz & P.L. McCall (eds), *Biotic interactions in recent and fossil benthic communities*: 431-478. New York: Plenum.

Cowen, R. 1988. The role of algal symbiosis in reefs through time. *Palaios* 3: 221-227.

Crowley, S.F. & Taylor, P.D. 2000. Stable isotope composition of modern bryozoan skeletal carbonate from the Otago Shelf, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 34: 331-351.

Cuffey, R.J. 1970. Bryozoan-environment interrelationships - an overview of bryozoan paleoecology and ecology. *Pennsylvania State University Earth and Mineral Sciences* 39: 41-45, 48.

Cuffey, R.J. & Fonda, F.S. 1976. "Giant" *Schizoporella*; sedimentologically important cheilostome bryozoans in Pleistocene and Recent carbonate environments of Bermuda, the Bahamas, and Florida. *Geological Society of America, Abstracts with Programs* 8(4): 474-475.

Easton, W.H. 1944. Review of Condra and Elias, "Study and revision of *Archimedes* (Hall)". *Journal of Paleontology* 18: 405-409.

Erez, J. 1978. Vital effect on stable-isotope composition seen in foraminifera and coral skeletons. *Nature* 273: 199-202.

Forester, R.M., Sandberg, P.A. & Anderson, T.F. 1973. Isotopic variability of cheilostome bryozoan skeletons. In G.P. Larwood (ed.), *Living and Fossil Bryozoa*: 79-94. London: Academic Press.

Gruszczynski, M., Halas, S., Hoffman, A. & Malkoski, K. 1989. A brachiopod calcite record of the oceanic carbon and oxygen isotope shifts at the Permian/Triassic transition. *Nature* 337: 64-68.

Haas, O. 1945. Notes on *Archimedes* [Glen Dean fm., Indiana]. *American Museum Novitates* 1945: 1-5.

Håkansson, E. 1979. Carboniferous to Tertiary development of the Wandel Sea Basin, Peary Land, eastern North Greenland. *Rapport Grønlands Geologiske Undersøgelse* 88: 73-83.

Håkansson, E., Heinberg, C. & Stemmerik, L. 1981. The Wandel Sea Basin from Holm Land to Lockwood Ø, eastern North Greenland. *Rapport Grønlands Geologiske Undersøgelse* 106: 47-63.

Håkansson, E. & Madsen, L. 1991. Symbiosis - a plausible explanation of gigantism in Permian trepostome bryozoans. In F.P. Bigey & J.-L. d'Hondt (eds), *Bryozoaires Actuales et Fossiles*: 151-159. Nantes: Societe des Sciences Naturalles de l'Ouest de la France. Memoire hors serie.

Håkansson, E. & Stemmerik, L. 1984. Wandel Sea Basin - the North Greenland equivalent to Svalbard and the Barents Shelf. In A.M. Spencer (ed.), *Petroleum Geology of the North European Margin*: 97-107. London: Graham and Trotman.

Håkansson, E. & Stemmerik, L. 1989. Wandel Sea Basin - a new synthesis of the late Paleozoic to Tertiary accumulation in North Greenland. *Geology* 17: 683-686.

Håkansson, E. & Stemmerik, L. 1995. Wandel Sea basin; basin analysis; a summary. *Rapport Grønlands Geologiske Undersøgelse* 165: 42-48.

Hallock, P. 1981. Algal symbiosis: a mathematical analysis. *Marine Biology* 62: 249-255.

Hallock, P. 1996. The energetics of algal symbiosis: implications for evolution. *Sixth North American paleontological convention; abstracts of papers*. Special Publication of the Paleontological Society 8: 158.

Hayasaka, I. & Hayasaka, S. 1953. Fossil assemblages of mollusks and brachiopods of unusually large sizes from the Permian of Japan. *Transactions and Proceedings of the Palaeontological Society of Japan* 10: 37-44.

Houston, R.M., Huber, B.T. & Spero, H.J. 1999. Size-related isotopic trends in some Maastrichtian planktic foraminifera: methodological comparisons, intraspecific variability, and evidence for photosymbiosis. *Marine Micropaleontology* 36: 169-188.

James, N.P., Bone, Y., von der Borch, C. & Gostin, V.A. 1992. Modern carbonate and terrigenous clastic sediments on a cool water, high-energy, mid-latitude shelf: Lacepede, southern, Australia. *Sedimentology* 39: 877-903.

Kaselowsky, J., Scholz, J. & Levit, G.S. 2002. The biological potential of encrusting bryozoans. *Senckenbergiana Lethaea* 82: 181-192.

Key, M.M., Jr., Thrane, L. & Collins, J.A. 2002. Functional morphology of maculae in a giant ramose bryozoan from the Permian of Greenland. In P.N. Wyse Jackson, C.J. Buttler & M.E. Spencer Jones (eds), *Bryozoan Studies 2001*: 163-170. Lisse, A.A. Balkema Publishers.

Lee, J.J. & Anderson, O.R. 1991. Symbiosis in foraminifera. In J.J. Lee & O.R. Anderson (eds), *Biology of Foraminifera*: 157-220. London: Academic Press.

Lee, J.J., McEnery, M.E., Kahn, E.G. & Schuster, F. 1979. Symbiosis and the evolution of larger foraminifera. *Micropaleontology* 25: 118-140.

Lowenstam, H.A. & Epstein, S. 1954. Paleotemperatures of the post-Aptian Cretaceous as determined by the oxygen isotope method. *Journal of Geology* 62: 207-248.

Lutaud, G. 1965. Sur la presence des microorganismes specifiques dans les glandes vestibulaires et dans l'aviculaire de *Palmicellaria skenei* (Ellis et Solander) Bryozoaire Chilostome. *Cahiers de Biologie Marine* 6:181-190.

Machiyama, H., Yamada, T., Kaneko, N., Iryu, Y., Odawara, K., Asami, R., Natsuda, H., Mawatari, S., Bone, Y. & James, N.P. 2002. Carbon and oxygen isotopes of cool-water bryozoans from the Great Australian Bight and their paleoenvironmental significance. In A.C. Hine, D.A. Feary & M.J. Malone (eds), *Proceedings of the Ocean Drilling Program, Scientific Results* 182: 1-29.

MacMunn. C.A. 1887. Chromatology of sponges. *Proceedings of the Physiological Society (London)* 3: xi-xii.

Madsen, L. 1987. Growth and polypide morphology in some ramose trepostome bryozoans from the Permo-Carboniferous of the Arctic. In J.R.P. Ross (ed.), *Bryozoa: Present and Past*: 169-176. Bellingham: Western Washington University Press.

Madsen, L. 1991. *The species concept in trepostome bryozoans - a study of phenotypical and genotypical variability within the genus* Tabulipora *in North Greenland*. Copenhagen: Unpublished Ph.D. dissertation, University of Copenhagen.

Madsen, L. 1994. Bryozoans from the Upper Palaeozoic Sequence in the Wandel Sea Basin, North Greenland. *Wandel Sea Basin: Basin Analysis Scientific Report* 6: 1-18.

Madsen, L. & Håkansson, E. 1989. Upper Palaeozoic bryozoans from the Wandel Sea Basin, North Greenland. *Rapport Grønlands Geologiske Undersøgelse* 144: 43-52.

McConnaughey, T. 1989. ^{13}C and ^{18}O isotopic disequilibrium in biological carbonates: I. Patterns. *Geochimica et Cosmochimica Acta* 53: 151-162.

Mii, H.-S., Grossman, E.L. & Yancey, T.E. 1997. Stable carbon and oxygen isotope shifts in Permian seas of West Spitsbergen-global change or diagenetic artifact? *Geology* 25: 227-230.

Morton, B. 2000. The biology and functional morphology of *Fragum erugatum* (Bivalvia: Cardiidae) from Shark Bay, Western Australia: the significance of its relationship with entrained zooxanthellae. *Journal of Zoology* 251: 39-52.

Norris, R.D. 1996. Symbioses as an evolutionary innovation in the radiation of Paleocene planktic foraminifera. *Paleobiology* 22: 461-480.

Norris, R.D. 1998. Recongition and macroevolutionary significance of photosymbiosis in mollusks, corals, and foraminifera. In R.D. Norris & R.M. Corfield (eds), *Isotope Paleobiology and Paleoecology*: 68-100. Paleontological Society Papers. Vol. 4.

Oltmans, F. 1923. *Morphologie und Biologie der Algen*. 2nd revised edition. Jena: Fischer.

Pätzold, J., Ristedt, H. & Wefer, G. 1987. Rate of growth and longevity of a large colony of *Pentapora foliacea* (Bryozoa) recorded in their oxygen isotope profiles. *Marine Biology* 96: 535-538.

Pearse, V.B. & Muscatine, L. 1971. Role of symbiotic algae (zooxanthellae) in coral calcification. *Biology Bulletin* 141: 350–363.

Rahimpour-Bonab, H., Bone, Y., Moussavi-Harami, R. & Turnbull, K. 1997. Geochemical comparisons of modern cool-water calcareous biota, Lacepede Shelf, south Australia, with their tropical counterparts. In N.P. James & J.A.D. Clarke (eds), *Cool-Water Carbonates*: 77-91.

Tulsa: SEPM Special Publication No. 55.
Raizen, D.N., Cuffey, R.J. & Rockwell-Garland, L.A. 1999. Unusually large ("giant") bryozoan colonies in the Rodman Limestone at Bellefonte (Middle Ordovician, central Pennsylvania). *Geological Society of America, Abstracts with Programs* 31(5): 66.
Rao, C.P. 1993. Carbonate minerals, oxygen and carbon isotopes in modern temperate Bryozoa, eastern Tasmania, Australia. *Sedimentary Geology* 88: 123-135.
Rao, C.P. & Nelson, C.S. 1992. Oxygen and carbon isotope fields for temperate carbonates from Tasmania and New Zealand. *Marine Geology* 103: 273-286.
Romanek, C.S. & Grossman, E.L. 1989. Stable isotope profiles of *Tridacna maxima* as environmental indicators. *Palaios* 4: 402-413.
Ross, J.R.P. 1995. Permian Bryozoa. In P.A. Scholle, T.M. Peryt, & D.S. Ulmer-Scholle (eds), *The Permian of Northern Pangea. Vol. 1, Paleogeography, Paleoclimates, Stratigraphy*: 196-209. Berlin: Springer Verlag.
Ross, J.P. & Ross, C.A. 1962. Faunas and correlation of the late Paleozoic rocks of northeast Greenland, part IV, Bryozoa. *Meddelelser Om Grønland* 167: 1-65.
Scholle, P.A. 1995. Carbon and sulfur isotope stratigraphy of the Permian and adjacent intervals. In P.A. Scholle, T.M. Peryt, & D.S. Ulmer-Scholle (eds), *The Permian of Northern Pangea. Vol. 1, Paleogeography, Paleoclimates, Stratigraphy*: 133-149. Berlin: Springer Verlag.
Schopf, T.J.M. 1977. Patterns and themes of evolution among the Bryozoa. In A. Hallam (ed.), *Patterns of Evolution*: 159-207. Amsterdam: Elsevier.
Scotese, C.R. & Langford, R.P. 1995. Pangea and the paleogeography of the Permian. In P.A. Scholle, T.M. Peryt, & D.S. Ulmer-Scholle (eds), *The Permian of Northern Pangea. Vol. 1, Paleogeography, Paleoclimates, Stratigraphy*: 3-19. Berlin: Springer Verlag.
Shulga-Nesterenko, M.I. 1949. *Funktsionalnoe, filogeneticheskoe i stratigraficheskoe zhachenie mikrostruktury skeletnkh tkanej mshanok*. Trudy Akademiya Nauk SSSR Paleontologicheskogo Instituta, t. 23.
Smith, A.M. & Key, M.M., Jr. 2004. Controls, variation and a record of climate change in a detailed stable isotope profile from a single bryozoan skeleton. *Quaternary Research* 61: 123-133.
Smith, A.M., Nelson, C.S., Key, M.M., Jr. & Patterson, W.P. in press. Stable isotope values in modern bryozoan carbonate from New Zealand and implications for paleoenvironmental interpretation. *New Zealand Journal of Geology and Geophysics* 47.
Spero, H.J., Bijma, J., Lea, D.W. & Bemiz, B.E. 1997. Effect of seawater carbonate concentration on foraminiferal carbon and oxygen isotopes. *Nature* 390: 497-500.
Stanley, G.D. 2003. The evolution of modern corals and their early history. *Earth Science Reviews* 60: 195-225.
Stanley, G.D. & Swart, P.K. 1995. Evolution of the coral-zooxanthellae symbiosis during the Triassic: a geochemical approach. *Paleobiology* 21: 179-199.
Stemmerik, L. 1995. Permian history of the Norwegian-Greenland Sea area. In P.A. Scholle, T.M. Peryt, & D.S. Ulmer-Scholle (eds), *The Permian of Northern Pangea. Vol. 2, Sedimentary Basins and Economic Resources*: 98-118. Berlin: Springer Verlag.
Stemmerik, L. 1997. Permian (Artinskian-Kazanian) cool-water carbonates in North Greenland, Svalbard and the western Barents Sea. In N.P. James & J.A.D. Clarke (eds), *Cool-Water Carbonates*: 349-364. Tulsa: SEPM Special Publication No. 55.
Stemmerik, L., Dalhoff, F., Larsen, B.D., Lyck, J., Mathiesen, A. & Nilsson, I. 1998. Wandel Sea Basin, eastern North Greenland. *Geology of Greenland Survey Bulletin* 180: 55-62.
Stemmerik, L. & Håkansson, E. 1989. Stratigraphy and depositional history of the Upper Palaeozoic and Triassic sediments in the Wandel Sea Basin, central and eastern North Greenland. *Rapport Grønlands Geologiske Undersøgelse* 143: 21-45.
Stemmerik, L., Håkansson, E., Madsen, L., Nilsson, I., Piasecki, S., Pinard, S. & Rasmussen, J.A. 1996. Stratigraphy and depositional evolution of the Upper Palaeozoic sedimentary succession in eastern Peary Land, North Greenland. *Bulletin Grønlands Geologiske Undersøgelse* 171: 45-71.
Stemmerik, L. & Magaritz, M. 1989. Carbon isotope variations in the Upper Carboniferous-Permian Mallemuk Mountain Group, eastern North Greenland. *Bulletin of the Geological Society of Denmark* 37: 205-211.
Stemmerik, L. & Worsley, D. 1995. Permian history of the Barents Shelf area. In P.A. Scholle, T.M. Peryt, & D.S. Ulmer-Scholle (eds), *The Permian of Northern Pangea. Vol. 2, Sedimentary Basins and Economic Resources*: 81-97. Berlin: Springer Verlag.
Taylor, P.D. 1999. Bryozoans. In E. Savazzi (ed.), *Functional Morphology of the Invertebrate Skeleton*: 623-646. London: John Wiley and Sons.
Taylor, P.D. & Voigt, E. 1999. An unusually large cyclostome bryozoan (*Pennipora anomalopora*) from the Upper Cretaceous of Maastricht. *Bulletin de l'Institut Royal des Sciences Naturalles de Belgique, Sciences de la Terre* 69: 165-171.
Trench, R.K. 1993. Microalgal-invertebrate symbiosis: a review. *Endocytobiosis Cell Research* 9: 135-175.
Veizer, J., Ala, D., Azmy, K., Bruckschen, P., Buhl, D., Bruhn, F., Carden, G.A.F., Diener, A., Ebnath, S., Godderis, Y., Jasper, T., Korte, C., Pawellek, F., Podlaha, O.G. & Strauss, H. 1999. $^{87}Sr/^{86}Sr$, $\delta^{13}C$ and $\delta^{18}O$ evolution of Phanerozoic seawater. *Chemical Geology* 161: 59-88.
Vogel, K. 1975. Endosymbiotic algae in rudists? *Palaeogeography, Palaeoclimatology, Palaeoecology* 17: 327-332.
Weber, J.N. & Woodhead, P.M.J. 1970. Carbon and oxygen isotope fractionation in the skeletal carbonate of reef-building corals. *Chemical Geology* 6: 93-117.
Weber, J.N. & Woodhead, P.M.J. 1972. Temperature dependence of oxygen-18 concentration in reef coral carbonates. *Journal of Geophysical Research* 77: 463-473.
Wefer, G. & Berger, W.H. 1991. Isotope paleontology: growth and composition of extant calcareous species. *Marine Geology* 100: 207-248.
Wurster, C.M., Patterson, W.P. & Cheatham, M.C. 1999. Advances in micromilling techniques: a new apparatus for acquiring high-resolution oxygen and carbon stable isotope values and major/minor elemental ratios from accretionary carbonate. *Computers & Geosciences* 25: 1159-1166.
Ziegler, A.M., Hulver, M.L. & Rowley, D.B. 1997. Permian world topography and climate. In I.P. Martin (ed.), *Late Glacial and Postglacial Environmental Changes*: 111-142. New York: Oxford University Press.
Zirpolo, G. 1923. Nuovo caso di simbiosi fra Zooxantelle e *Bicellaria ciliata* briozoo del Golfo di Napoli. *Rendiconti della Assemblea Ordinaria e del Convegno dell'Unione Zoologica Italiana in Genova.* Naples: Zoologico Italiano.