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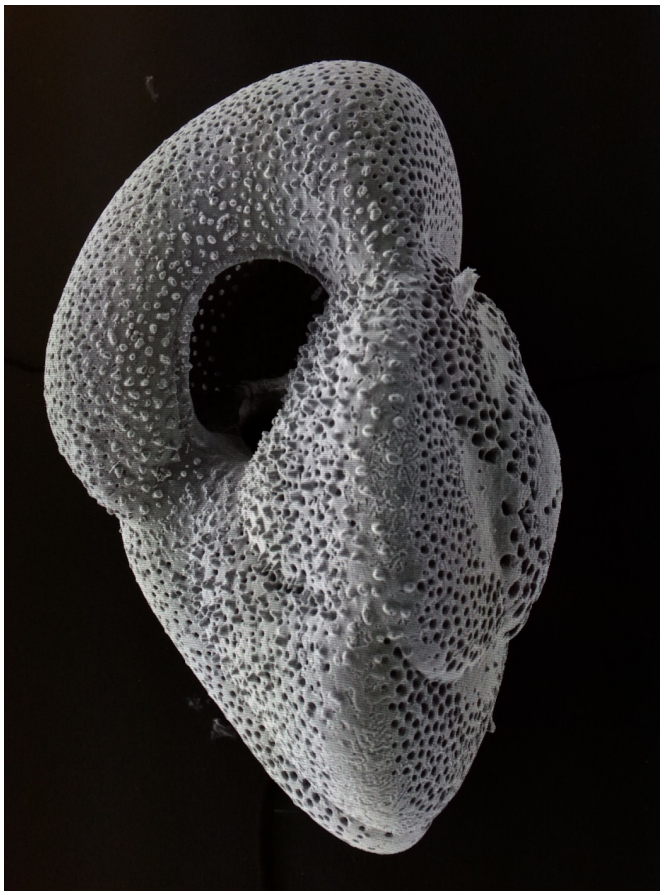
Evidence of photosymbiosis prior to the Eocene-Oligocene climate transition?

Size-related stable isotope trends in some late Eocene planktonic foraminifera

Anna Nyberg



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Abstract

Studies on photosymbiotic planktonic foraminifera from late Eocene are very few, and it has not been predicted that a planktonic microfossil from that time live in symbiosis with photosynthesizing algae. It is most essential to the paleoecologists studying the paleoclimate to get accurate $\delta^{13}C$ isotopic values in the carbon cycling system when they look into the marine carbon pump which are essential for future climate studies. But the foraminifera living in symbiosis provide an offset of $\delta^{13}O$ from the ambient DIC (dissolved inorganic carbon) in the ocean. In this study I have examined some late Eocene planktonic foraminifera specimen in different test size by using the $\delta^{13}C$ and $\delta^{18}O$ stable isotope analysis to find out if any of the specimen exhibit evidence of photosymbiosis.

Keywords

Planktonic foraminifera, photosynthesizing algae, photosymbiosis, Eocene, stable isotope, metabolic effect, recrystallization, calcification.

Contents

Introduction

For a long time it has been an assumption that $\delta^{13}C$ and $\delta^{18}O$ in calcite producing organisms such as planktonic foraminifera provide us with information about the sea temperature and nutrient condition in the ocean. There is a precondition that the foraminifera isotopic records are in equilibrium with the seawater from which the calcite shell was made, (under the condition that the nutrients content, mixing water or carbon cycle gets undisturbed). However, other processes may occur that can change the foraminifera test isotopic composition so it will starts to show differences from the seawater. One is that some planktonic foraminifera species have symbiotic associations with photosynthesizing algae which results in a positive offset of $\delta^{13}C$ approximately 1 ‰ from ambient sea water values (Birch et al. 2012 and D´Hondt et al. 1994). Studies in modern planktonic foraminifera show that this is controlled by the algal symbionts living on the foraminifera and fractionating out the ^{12}C (light carbon isotope) leaving the carbon pool available for calcification relatively enriched in ^{13}C (the heavier carbon isotope) (Spero & Deniro, 1987 and Spero & Lea, 1993). This is known as the symbiosis carbon vital effect (Spero & Lea, 1993). It is important to identify conditions from the past about the nutrients supply and ocean mixing (stratification in the water column) to get a better understanding of ocean carbon pumping through time, which is relevant to our climate in the future (Birch et al. 2012). But due to the vital effects $\delta^{13}C$ from the foraminifera can be misleading because symbiotic species will record a higher value of $\delta^{13}C$ in the real surface water DIC (dissolved inorganic carbon) values of seawater (Spero & Lea, 1993 and Birch, et al. 2012).

Previous studies on photosymbiosis of different planktonic foraminifera from Cretaceous, Paleocene, early and middle Eocene show similar $\delta^{13}O$ isotopic signals that indicate photosymbiosis in some species (D´Hondt et al. 1994). Two large genera that lived in symbiosis were *Morozovella* and *Acarinina,* but most of the species in these genera went extinct during the middle of Eocene (Pearson et al. 2006). There are very few studies on the late Eocene planktonic foraminifera living in symbiosis and it is still not clear if any of the species was symbiotic (Coxall personal communication).

The goal with this project is to use $\delta^{13}C$ and $\delta^{18}O$ measurements in late Eocene planktonic foraminifera to investigate their paleoecologies and establish if some species from late Eocene show evidence for photosymbiosis. This may be of use to scientist working on the carbon cycling in the water column so they will know what species show $\delta^{13}C$ pattern typical of photosymbiosis, so that they can be avoided. The samples come from two deep sea drill sites in the Atlantic Ocean, ODP Site 1053B from Blake Nose and IODP Site U1411 on the Newfoundland margin. The purpose by looking at two Sites (with different sediment composition) is to compare the isotopic signals from these two different paleoecological environments.

If the expectations from $\delta^{13}C$ and $\delta^{18}O$ isotopic signals in the foraminifera from these two sites is showing any kind of symbiotic evidence. Then can these specimens be avoided for the paleoecological research of carbon cycling related to climate studies. This is the Hypothesis.

Geological settings and Site details

In this project samples (in order: Site/core/section/depth in cm) has been taken from 1053B/5H/3 (128-130), 1053B/4H/5 (72-74), 1053B/3H/1 (25-27) Blake Nose. U1411B/19H/2 (94,5-96), U1411B/19H/4 (134,5-136) and U1411B/20H/4 (58,5-60) New Foundland margin.
Site 1053 is part of a transect in leg 171B with a water depth of 1293-2586 mbsl (meter below sea level) and is located north east of Florida. 1053B is drilled at 1630 mbsl and 182.4 mbsf (meter bellow the seafloor). The main purpose to drill in this area was to recover a thick layer of Eocene sediment for further investigation about the structure on the deep water and climate events during the Paleogene. The accumulation rate of the layer on top had been very small in this area and most of Oligocene and Miocene layer had eroded away which left a few meters of sediment covering the Eocene layer. The Eocene layer consist mostly of nannofossil calcareous ooze, siliceous ooze and chalk with miner content of foraminifera which exhibit recrystallisation pattern of being milky white and having frosty texture (Norris et al. 1998). Recrystallization commonly occur in deep buried calcareous rich sediment and chalks where chemical interactions with pore water can occur. Whereas in clay rich sediment the pore water has harder to penetrate and do not interact with the calcite in foraminifera, so their shell will exhibit a better preservation (Sexton, Wilson, & Pearson, 2006).

Appendix. 1. the top image show the location of Blake Nose, north east of Florida. Location of this site 1053B is: 29°59.5391´N, 76°31.4141´W. Picture is taken from: Ocean Drilling Program, Leg 171B preliminary report, Blake Nose (Norris et al. 1997)**, and below is Site U1411B which is located southeast from Newfoundland in Newfoundland margin. Picture is taken from:** Expedition 342 Scientists (2012).

Site U1411 is located northwest of the Atlantic 41°37.0993´N, 48°59.9839´W and is also a part of a transect in leg 342. U1411B is drilled at 3298 mbsl and the core penetrates 254,2 m through the sediment. Two of the major reason why they drilled in this area was to get a large thick clay-rich layer enriched of microfossil and to get a good reconstruction of the CCD (carbon compensation depth) through Paleogene. Most of the Eocene layer consist of nannofossil clay and ooze with well preserved microfossil. Most of the foraminifera are transparent, have glassy texture, are none filled and has not gone through recrystallization.
(Expedition 342 Scientists (2012).

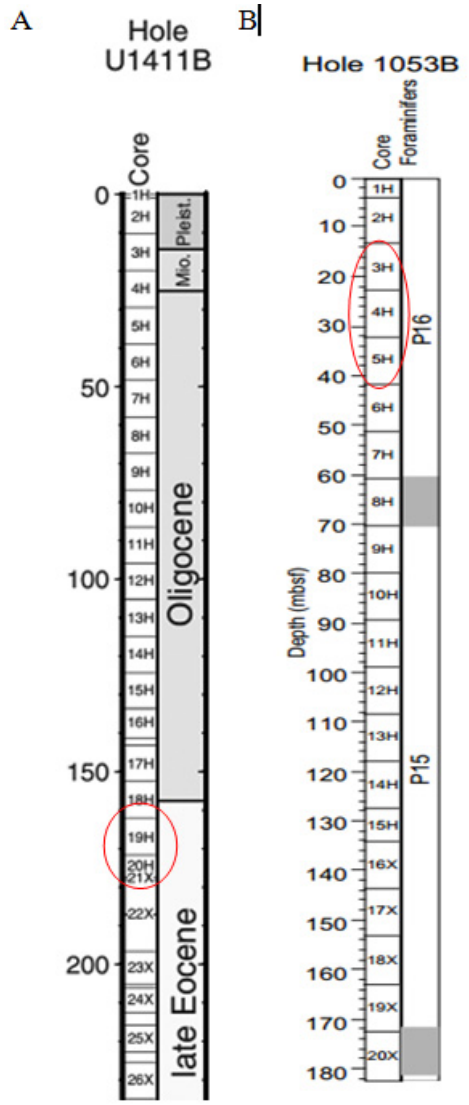


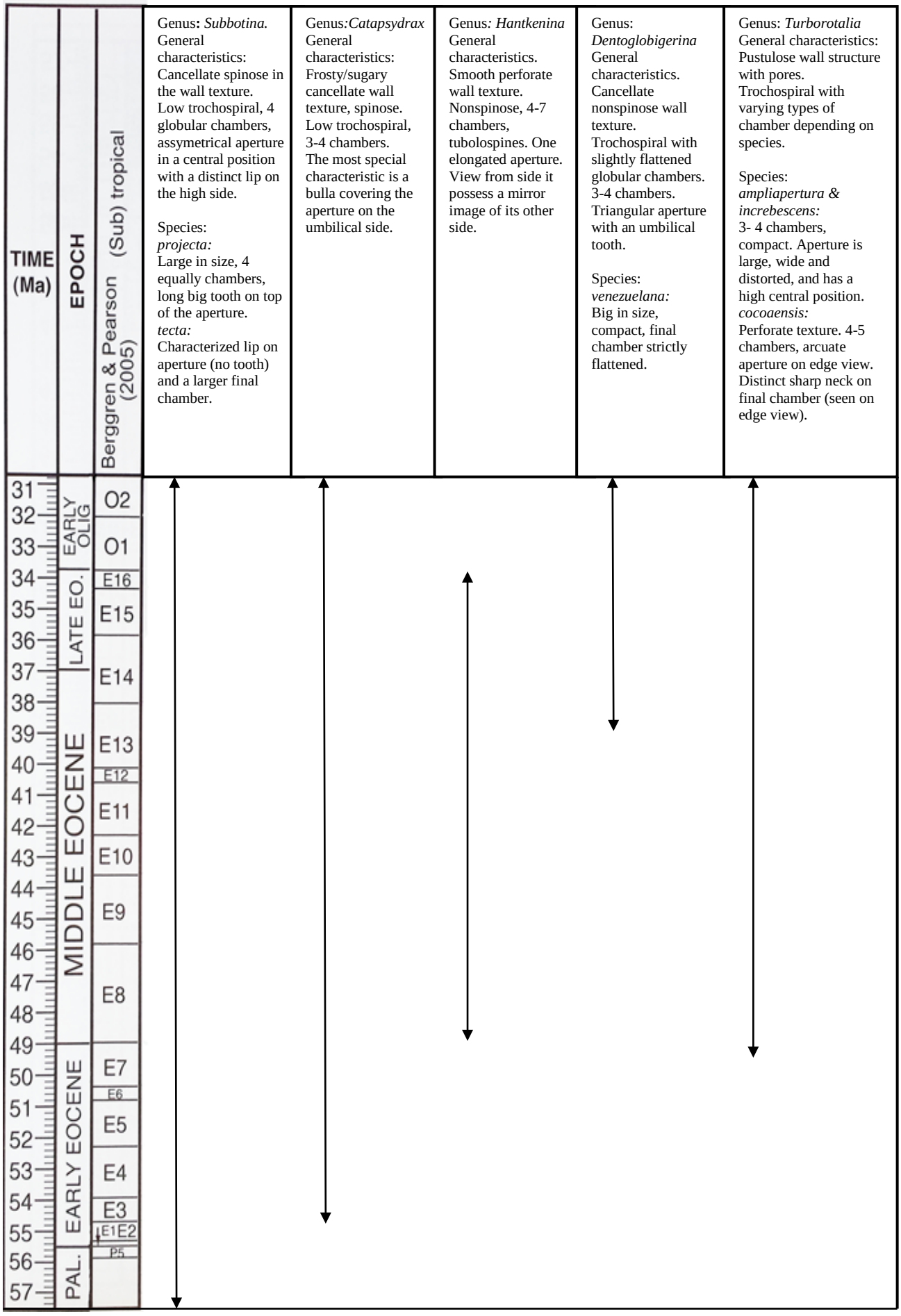
Fig. 1
Samples have been picked from cores in the red circled area showing biozone in late Eocene. (A) Site U1411 (Newfoundland margin), (B) Site 1053 (Blake Nose). In site 1053 the biozone P16 represent biozone E15 and the border to E16 of late Eocene according to Berggren and others (1995) from the Atlas of Eocene planktonic foraminifera (Pearson, et al. 2006) **Pictures is taken from** Expedition 342 Scientists (2012) and (Norris et al. 1998).

In this study I have been following the taxonomy of Pearson et al. 2006. In the Atlas of Eocene Planktonic Foraminifera and some updates of species from the forthcoming Oligocene Atlas of Planktonic Foraminifera (Paleogene Foraminifera Working Group, Wade et al., which are in preparation).

Background information about the ecology and taxonomy

Planktonic foraminifera consist of a single cell. They have one or often several chambers and the living micro organism possess protruding spines and pseudopodia which they use to catch their food with. It is also in the protruding spines where the symbionts live inside if there are symbiotic living organisms in a particular species (this is the characteristics of modern photosymbiotic living planktonic foraminifera). But in the past there has also been non spinose planktonic foraminifera living in symbiosis (D´Hondt et al. 1994 and Armstrong & Brasier, 2005). Between the chambers there is an aperture. It is mostly located on the umbilical side. The majority of the planktonic foraminifera have one aperture, but some possess two or more (genus: *Globigerinatheka*, for instance) (Armstrong & Brasier, 2005). All the selected species in this study have only one visible aperture.

The morphology in some species show similar feature and it can be hard to distinguish from one another. A spiral, a chamber, an aperture or position of a tooth may not be the critical feature to name a foraminifera. Sometimes there need to be more analysis to get the right morphology to distinguish one species from another for instance by looking at the wall structure in SEM (Pearson et al. 2006).
The wall structure is built up of $CaCO_3$ and in the SEM it is possible to se including tiny *coccoliths,* pores, texture on the wall surface and evidence of recrystallization.
The morphology in species changes through time gradually but differs in rates depending on the specimen and the environment (this is very usal in the planktonic foraminifera) and by therefore new species occur. This change in the morphology ecology is evidence of evolution. Sometimes around extinction events a more abrupt change can occur and different specimen/populations disappears totally and are replaced by new species with another morphology (Armstrong & Brasier, 2005).
Planktonic foraminifer lives in the upper surface water (surface mized layer ~100-200 m), but some of them can migrate down to approximately 500-1000 m depth (depending on species). They collect information about seatemperature and salinity from the oxygen stable isotope $\delta^{18}O$, which make them perfect to reconstruct the ocean water temperature and condition in the past and can be used as a climate indicator when studing paleoclimate (Armstrong & Brasier, 2005 and Birch et al. 2013).


TIME (Ma)
EPOCH
(Sub) tropical Berggren & Pearson (2005)
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EARLY OLIG
LATE EO.
MIDDLE EOCENE
EARLY EOCENE
PAL.
O2
O1
E16
E15
E14
E13
E12
E11
E10
E9
E8
E7
E6
E5
E4
E3
E1E2
P5
Genus: *Subbotina.* General characteristics: Cancellate spinose in the wall texture. Low trochospiral, 4 globular chambers, assymetrical aperture in a central position with a distinct lip on the high side.
Species: *projecta:* Large in size, 4 equally chambers, long big tooth on top of the aperture. *tecta:* Characterized lip on aperture (no tooth) and a larger final chamber.
Genus:*Catapsydrax* General characteristics: Frosty/sugary cancellate wall texture, spinose. Low trochospiral, 3-4 chambers. The most special characteristic is a bulla covering the aperture on the umbilical side.
Genus*: Hantkenina* General characteristics. Smooth perforate wall texture. Nonspinose, 4-7 chambers, tubolospines. One elongated aperture. View from side it possess a mirror image of its other side.
Genus: *Dentoglobigerina* General characteristics. Cancellate nonspinose wall texture. Trochospiral with slightly flattened globular chambers. 3-4 chambers. Triangular aperture with an umbilical tooth.
Species: *venezuelana:* Big in size, compact, final chamber strictly flattened.
Genus: *Turborotalia* General characteristics: Pustulose wall structure with pores. Trochospiral with varying types of chamber depending on species.
Species: *ampliapertura & increbescens:* 3- 4 chambers, compact. Aperture is large, wide and distorted, and has a high central position. *cocoaensis:* Perforate texture. 4-5 chambers, arcuate aperture on edge view. Distinct sharp neck on final chamber (seen on edge view).

Fig. 2
General taxonomy of the genera from the selected species in this study. Also showing time scale and the biozone E15 from which they are collected from. The solid line represent the evolution of the genera (*S. projecta* and *S. tecta* are new species and has not yet a precise evolving date).
The biozone and time scale are collected from the Atlas of Eocene Planktonic Foraminifera (Pearson et al. 2006).

The ecology differs from species to species and depends on the temperature, possition in the watercolumn and nutrient availibility. It has been established that species living in shallower warmer water shows a more negative $\delta^{18}O$ trend than those organism living deeper down the water column. This method has also be applied to fossil species. One example tested is the Eocene genus *Subbotina* (thought to be non symbiotic living organism) which is showing more positive $\delta^{18}O$ than *Morozovella* (thought to be symbiotic living). and is therefore suggesting in living deeper down the water column. (Armstrong & Brasier 2005 and Pearson et al. 2006).
In late eocene some of the selected species for this project lived deeper down the watercolumn (*Dentoglobigerina*, *Subbotina* and *Catapsydrax*) And some of them may have begin there life cycle in the surface water and by time migrate down the water column (Pearson et al. 2006).

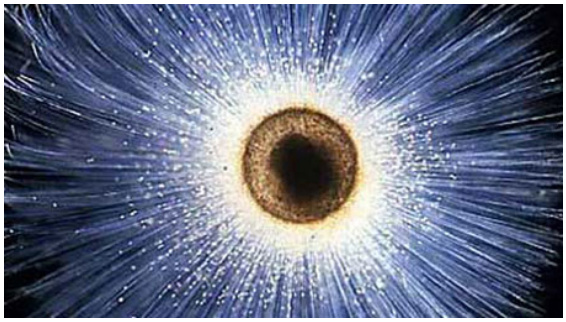


Fig. 3 *Orbulina universa*
Modern living single cell photosymbiotic planktonic foraminifera with characterized spines surrounding the microorganism. Picture is taken by Dr. Howard J Spero.

The biostratigraphy of foraminifera is divided into different biozones based on the biozone foraminifera index which is the content of a specific specimen existing in this zone and not in the next following zone. Representing the late Eocene the selected species in this project are picked from biozone E15, containing *Globigerinatheka index.* This species do not excist in the zone afterwards (Pearson et al. 2006). The next coming zone E 16 ends at Eocene/Oligocene boundary and is characterised by the extinction of all remaining Hantkenina species and including *T.cocoaensis* (Wade & Pearson, 2008).

Photosymbiosis in living and fossil planktonic foraminifera, modern observations and isotopic signals

The biggest purpose of studying marine microfossil is the evolutionary perspectives of abundance, when, where and how did the fossil lived and how long did the fossil record last for each specimen? In their $CaCO_3$ shell they also collect information about the environment they live in, which can be clear in the isotopic analysis of $\delta^{13}C$ and $\delta^{18}O$. This information is important for scientist to use when to map and identify events from the past and use the information to understand the future (Armstrong & Brasier, 2005 and Birch et al. 2012). In the marine research it is most helpful to map the microfossil pattern to understand nutrition contents and organism migration, surface and deep water circulation, carbon exchange and DIC, temperature changes and changes in

climate (Armstrong & Brasier, 2005 and Wade & Pearson, 2008).

Why is the studying of photosymbiosis of relevance?

If scientist want to look at carbon cycling in the water column in the past it is important to know about $\delta^{13}C$ deviations from DIC (dissolved inorganic carbon) in the ocean. As mentioned the $CACO_3$ shell of the planktonic foraminifera collect information about their habitat in the ocean and lives mostly in equilibrium with the surrounding environmental conditions (Birch et al. 2012). When a species lives in symbiosis with a photosynthesizing algae it will provide more of the ^{13}C to the shell calcification (because the phytoplankton use the ^{12}C in the photosynthetic activity) and by therefore give small inaccuracy in the isotopic signals about the inorganic carbon content in the water column (Norris,1996).

Cultivations have been done in laboratory of living planctonic species that exhibit photosymbiotic pattern. By comparing the isotopic signals in the results from these individuals to fossil taxa which shows similar isotopic deviations, it is possible to get a picture of photosymbiosis in the past (Spero & Lea, 1993). Because the fossil record doesn´t contain any algal symbionts, just the isotopic signatures are preserved (Wade et al. 2008).

In the past *Morozovella* and *Acarinina* show significant symbiotic pattern (Birch et al. 2012 and Norris, 1996). The symbiosis in these individuals slowly ceased and finally they died out. The remaining *Morozovella* species went extinct in late middle Eocene followed by the remaining species of *Acarinina* shortly thereafter in the late Eocene (Wade et al. 2008 and Pearson et al. 2006). These two large genera of photosymbionts lived in shallow warm water through their whole life according to a negative and a relative consistant value in the $\delta^{18}O$ isotopic signal and a strong possitive $\delta^{13}C$ signal (Norris, 1996).

Living planktonic foraminifera have dinoflagellates or chrysophytes as symbionts (photosynthesizing algaes). Foraminifera gets fixed carbon from the symbionts that they use as an energy source for instance in their calcification process. In return the foraminifera provide the symbionts with protection and nutrients from food leftovers (Norris, 1996).

The photosymbiosis can be affected by the ontogenetic condition (stage between juvenile and adult) because some species change their position in depth and migrate through the water column to different habitat during their life cycle.

The intensity of symbiosis also differs from one species to another and that can also have an influence on the result. Increasing size also increases the symbiosis, and the intensity of the symbioses can be even stronger with increasing density. (Birch et al. 2012 and Wade et al. 2008). Species of *Morozovella* and *Acarinina* show a strong intensity of symbiosis over a small range of fractions (approximately ~1‰ positive $\delta^{13}C$ over 200 µm range in size). Modern *Globigerinoides sacculifer* show a similar pattern. Small differences in the symbiosis intensity can depend on how much light that was availibly (where above the thermocline they lived) and nutrient content. Modern experiment in laboratory on these living specimen of *G. sacculifer* has been done and it has been found that there are a strong possitive correlation between test size and more possitive values of $\delta^{13}C$ in surface living planktonic foraminifera. This experiment show an relationship between increasing size and $\delta^{13}C$ values towards light intensity that is linked to a photosymbiotic ecology (Norris, 1996 and Spero & Lea, 1993).

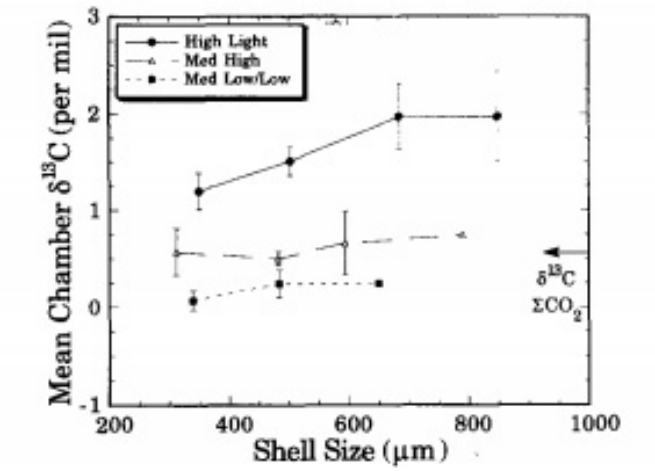


Fig. 4
This image show the relationship between increasing $\delta^{13}C$ and shell size against different light intensity on the cultivating *G. sacculifer* from laboratory research. Pickture is taken from (Spero & Lea, 1993).

Similar signatures have also been found in fossil species of *Morozovella* and *Acarinina* and this is an essential indicator of photosymbiosis (Norris, 1996).

Expectations from the isotopic analysis

Trend signals of $\delta^{18}O$ show an increasing pattern through ontogenetic life cycle in some species which suggest that these species migrate through the water column during their life beginning in shallow warmer water (Houston & Huber, 1998). There are small characteristics in the stable isotope that show photosymbiotic signatures which can be very useful when to interpret the results in this study.
Some of the most distinctive pattern for the photosymbiotic are: (1) $\delta^{18}O$ show the most negative values in the photosymbiotic species because the photosymbiosis occur in the shallow surface water of the photic zone, (2) $\delta^{18}O$ maintain relatively consistent values with increasing size in the foraminifera and (3) $\delta^{13}O$ exhibit an positive trend of approximately 0,5-1,0 ‰ with increasing shell size (that is why it is important to get a large examined range of test size fractions for each species) (Norris, 1996). Small changes in the $\delta^{18}O$ to positive trends in the shell can depend on migration to different habitats in the upper water column (Houston & Huber, 1998). The phytoplankton prefers the ^{12}C isotope because it is lighter and more energy can be achieved during the photosynthesis, which will leave a higher content of ^{13}C in the living microorganism (Armstrong & Brasier, 2005). If the *Dinoflagellates* or *chrysophytes* are living in symbiosis with the planktonic foraminifera it will leave more positive values of $\delta^{13}C$ in a photosymbiotic species for ^{13}C to be used in the calcification process (Norris, 1996). There are also increasing values of the $\delta^{13}C$ stable isotope depending of a metabolic effect. This normally shows an increasing pattern in the smaller size and then flattens out in the larger size fractions (Birch et al. 2012 and D´Hondt et al. 1994).
Site 1053B has been exposed to recrystallisation and that can have an affect on the isotopic results (D´Hondt et al. 1994). Which are shown in the result and will be further discussed.
It is also important to emphasize that in this study, *Turborotalia ampliapertura* and *Turborotalia increbescens* is in a mixture because the oxygen and the carbon in their shell have a very similar content (Wade & Pearson, 2008).

Method and materials

The assemblage of species that has been picked out (from each site and core) and used in this study is: *Catapsydrax unicavus, Dentoglobigerina venezuelana, Subbotina projecta/ Subbotina tecta,*

Turborotalia cocoaensis, Turborotalia ampliapertura and *Turborotalia increbescens. Hantkenina* has also been picked out, but all the samples exhibited too few specimen of the *Hantkenina* that they could not be used for isotopic analysis. All the samples are collected from late Eocene in biozone E15 on the border to E16 before the Eocene/Oligocene boundary.

The sediment containing micro fossils, from site 1053B and U1411B were picked out every 2 respective 1,5 cm depth interval through each section in the core. The samples were freeze dried, weighed in flasks and some mille Q H_2O was added (so the sediment could be dissolved) before the samples were put on a shaker table (175-180 RPM) for a couple of hours. After that the samples were washed with mille Q H_2O over 63 µm sieves and dried at 49,5° in an oven over the night.

The next day samples were weighed and sorted in vials and marked with Site/core/section and depth.

The core samples with planktonic foraminifera were sieved through 10 different size fraction sieves between 500-90 µm. Then each size fraction from each core was examined in a WILD M10 microscope and six different species were picked out from each fraction depending on the diversity in the specific fraction. All the species were weighed and put into 12 ml vials before examination and exposure in the isotopic analysis to find out if there might be some evidence of any photosymbiotic potential. The weight of different species is mainly around 0,2 mg but can varies a lot depending on content of species for each sample. Minimum weight for all the species in the isotopic analysis may not be less than 0,05 mg, because that can give a higher standard deviation in the isotopic analysis. All cores from each site are located in the E15 and some on the border to E16 biozone. (*Globigerinatheka index) near the Eocene/Oligocene boundary.*

Site 1053B

General this site contained foraminifera which were not so well preserved. Roughly estimated 95% of the apertures are filled, which lead to difficulties with some of the species to distinguish. A lot of dirt, salt crystals, mineral grains and also radiolarian are more abundant in the smaller fractions below 250 µm. Overall this site has a sparse amount of species and almost everything that was present was picked out and used in the isotopic analysis and SEM.

Core 5H/3 has a good preservation in the two lowest of fractions where the apertures are not filled. Core 4H/5 has the best diversity of foraminifera and a large amount of species compare with the other two cores (that was used in this study). This core presented a dominance of the genus *Turborotalia*. Compared to the other two cores that had more mixing of the dominant species where the genus of *Subbotina* and *Dentoglobigerina* were the most distinctive in different fractions. Core 3H/1 presented the worst diversity and amount of the selected species for this project and in some fractions there were quite hard to achieve enough weight for the isotopic analysis.

References of this information come from observation in the microscope.

Species	Size fraction intervall (µm)	Mean fraction (µm)	Number (n) of species used in analysis.				Sample weight for analysis in (mg)	
			1053B/5H/3 (n),	(mg)	1053B/4H/5 (n),	(mg)	1053B/3H/1 (n),	(mg)
Subbotina projecta	>500	500	2	0,162	-		-	
	<500 - >425	462,5	3	0,186	3	0,180	3	0,178
	<425 - >355	390	4	0,187	4	0,185	5	0,195
	<355 - >300	327,5	11	0,196	7	0,208	6	0,127
	<300 - >250	275	14	0,193	9	0,199	11	0,149
	<250 - >212	231	17	0,148	16	0,179	13	0,126
	<212 - >180	196	-		13	0,086	-	
	<180 - >150	165	-		-		-	
	<150 - >125	137,5	-		-		-	
	<125 - >90	107,5	-		-		-	
Dentoglobigerina venecuelana	>500	500	2	0,212	1	0,197	2	0,234
	<500 - >425	462,5	3	0,198	2	0,198	3	0,213
	<425 - >355	390	4	0,187	4	0,207	4	0,201
	<355 - >300	327,5	7	0,200	6	0,187	9	0,171
	<300 - >250	275	-		-		8	0,097
	<250 - >212	231	-		-		-	
	<212 - >180	196	-		-		-	
	<180 - >150	165	-		-		-	
	<150 - >125	137,5	-		-		-	
	<125 - >90	107,5	-		-		-	
Catapsydrax	>500	500	-		-		-	
	<500 - >425	462,5	-		-		-	
	<425 - >355	390	3	0,096	2	0,075	-	
	<355 - >300	327,5	7	0,190	7	0,203	6	0,148
	<300 - >250	275	11	0,207	10	0,185	8	0,153
	<250 - >212	231	9	0,091	14	0,184	8	0,104
	<212 - >180	196	9	0,048	17	0,106	-	
	<180 - >150	165	-		16	0,060	-	
	<150 - >125	137,5	-		-		-	
	<125 - >90	107,5	-		-		-	
Turborotalia cocoaensis	>500	500	-		2	0,230	-	
	<500 - >425	462,5	-		2	0,167	-	
	<425 - >355	390	3	0,119	3	0,167	-	
	<355 - >300	327,5	6	0,138	5	0,202	5	0,094
	<300 - >250	275	11	0,192	9	0,199	10	0,153
	<250 - >212	231	19	0,197	16	0,195	14	0,128
	<212 - >180	196	12	0,071	23	0,178	20	0,106
	<180 - >150	165	24	0,092	35	0,165	17	0,063
	<150 - >125	137,5	32	0,065	36	0,100	26	0,060
	<125 - >90	107,5	-		-		-	
Turborotalia ampliapertura/ increbescens	>500	500	-		-		-	
	<500 - >425	462,5	-		-		-	
	<425 - >355	390	-		-		-	
	<355 - >300	327,5	9	0,190	8	0,201	5	0,094
	<300 - >250	275	16	0,188	13	0,202	14	0,173
	<250 - >212	231	14	0,096	18	0,188	15	0,158
	<212 - >180	196	-		29	0,188	17	0,095
	<180 - >150	165	-		-		-	
	<150 - >125	137,5	-		-		-	
	<125 - >90	107,5	-		-		-	

Table 1.
Specimen from site 1053B (Blake Nose) that has been used in the isotopic analysis. Depending on the diversity in different cores the content varies a lot.
n = number of specimen that has been picked out for the analysis.
mg = the weight in milligram of each sample.

Species	Size fraction intervall (µm)	Mean fraction (µm)	Number (n) of species used in analysis.				Sample weight for analysis in (mg)	
			U1411B/19H/2 (n),	(mg)	U1411B/19H/4 (n),	(mg)	U1411B/20H/4 (n),	(mg)
Subbotina projecta	>500	500	-		-		-	
	<500 - >425	462,5	4	0,184	5	0,212	5	0,222
	<425 - >355	390	6	0,230	6	0,184	6	0,186
	<355 - >300	327,5	7	0,202	12	0,181	9	0,184
Subbotina tecta	<300 - >250	275	16	0,196	10	0,107	13	0,167
	<250 - >212	231	20	0,137	23	0,121	20	0,162
	<212 - >180	196	-		17	0,067	22	0,101
	<180 - >150	165	-		-		-	
	<150 - >125	137,5	-		-		-	
	<125 - >90	107,5	-		-		-	
Dentoglobigerina venecuelana	>500	500	2	0,158	3	0,207	2	0,170
	<500 - >425	462,5	4	0,213	4	0,198	3	0,185
	<425 - >355	390	5	0,181	6	0,217	4	0,197
	<355 - >300	327,5	8	0,179	9	0,211	10	0,194
	<300 - >250	275	12	0,133	-		-	
	<250 - >212	231	-		-		-	
	<212 - >180	196	-		-		-	
	<180 - >150	165	-		-		-	
	<150 - >125	137,5	-		-		-	
	<125 - >90	107,5	-		-		-	
Catapsydrax	>500	500	-		-		-	
	<500 - >425	462,5	-		-		-	
	<425 - >355	390	-		3	0,129	3	0,111
	<355 - >300	327,5	8	0,187	8	0,190	6	0,134
	<300 - >250	275	11	0,180	13	0,187	12	0,195
	<250 - >212	231	20	0,189	20	0,169	19	0,171
	<212 - >180	196	23	0,141	23	0,118	21	0,122
	<180 - >150	165	28	0,095	33	0,107	-	
	<150 - >125	137,5	-		-		-	
	<125 - >90	107,5	-		-		-	
Turborotalia cocoaensis	>500	500	-		-		-	
	<500 - >425	462,5	-		-		-	
	<425 - >355	390	5	0,133	4	0,100	6	0,203
	<355 - >300	327,5	8	0,190	10	0,187	8	0,183
	<300 - >250	275	12	0,176	14	0,199	13	0,193
	<250 - >212	231	15	0,136	14	0,111	20	0,182
	<212 - >180	196	17	0,090	19	0,100	27	0,144
	<180 - >150	165	-		22	0,070	33	0,118
	<150 - >125	137,5	-		35	0,067	37	0,089
	<125 - >90	107,5	-		-		-	
Turborotalia ampliapertura/ increbescens	>500	500	-		-		-	
	<500 - >425	462,5	-		-		-	
	<425 - >355	390	-		-		-	
	<355 - >300	327,5	5	0,081	6	0,094	5	0,092
	<300 - >250	275	9	0,104	15	0,173	13	0,155
	<250 - >212	231	12	0,079	24	0,158	15	0,105
	<212 - >180	196	-		23	0,095	15	0,062
	<180 - >150	165	-		-		-	
	<150 - >125	137,5	-		-		-	
	<125 - >90	107,5	-		-		-	

Table 2.
Specimen from site U1411B (Newfoundland margin) that has been used in the isotopic analysis. Depending on the diversity in different cores the content varies a lot.
n = number of specimen that has been picked out for the analysis.
mg = the weight in milligram of each sample.

Site U1411B

The general pattern of preservation in this site is that all specimen possess none filled apertures. The most dominant species in the larger fractions (>300 µm) is Dentoglobigerina and for the fraction below 300 µm, the most dominant species are Catapsydrax. This is general for all the examined cores in this project from this site.
Core 19H/2 and 19H/4 shows a similar pattern of preservation and similarity in species dominance in each fraction. In the lower fraction (<250 µm) the good preservation decreases immensely and broken shell fragment are getting quite abundant (roughly estimated to 30-40%). The bad preservation continues down the size fractions.
Core 20H/4 shows more diversity and is richer in foraminifer (more than 19H/2 and 19H/4). This core shows an unidentified species. Typical for this "new" species is that it consist of three rounded chambers with two large apertures one on umbilical side and one on the spiral side (this species is not part of this project so no pictures was taken). But to make a note, this species occurred somewhere between core 19H/4 and 20H4.
All the examined cores in this site exhibit secondary alteration of black spots on the shell structure, this is not so common in the larger fraction but getting more common in the lower fractions (approximately 10% of the content in fraction <250 µm) and increases further down the fractions up to 50-60 % in the two lowest of fractions. References of this information come from observation in the microscope.

SEM imaging

The species have been imaged using a QUANTA FEG 650 Scanning electron microscope (SEM) with secondary electron image topography (ETD). In SEM it is possible to see the calcite wall texture including recrystallization pattern of the shell structure in higher resolution from site 1053B.
The sample of species is attached on stubs. Samples gets an Au coating which remove the electrons and the sample gets unloaded. The samples are inserted into a vacuum chamber with high vacuum (near no particles in air) where electrons hits the gold coated foraminifer and take a picture. Most of the pictures have a scale bar of 100-400 µm beside the shell structures pictures that have much higher resolution (approximately 20-40 µm).

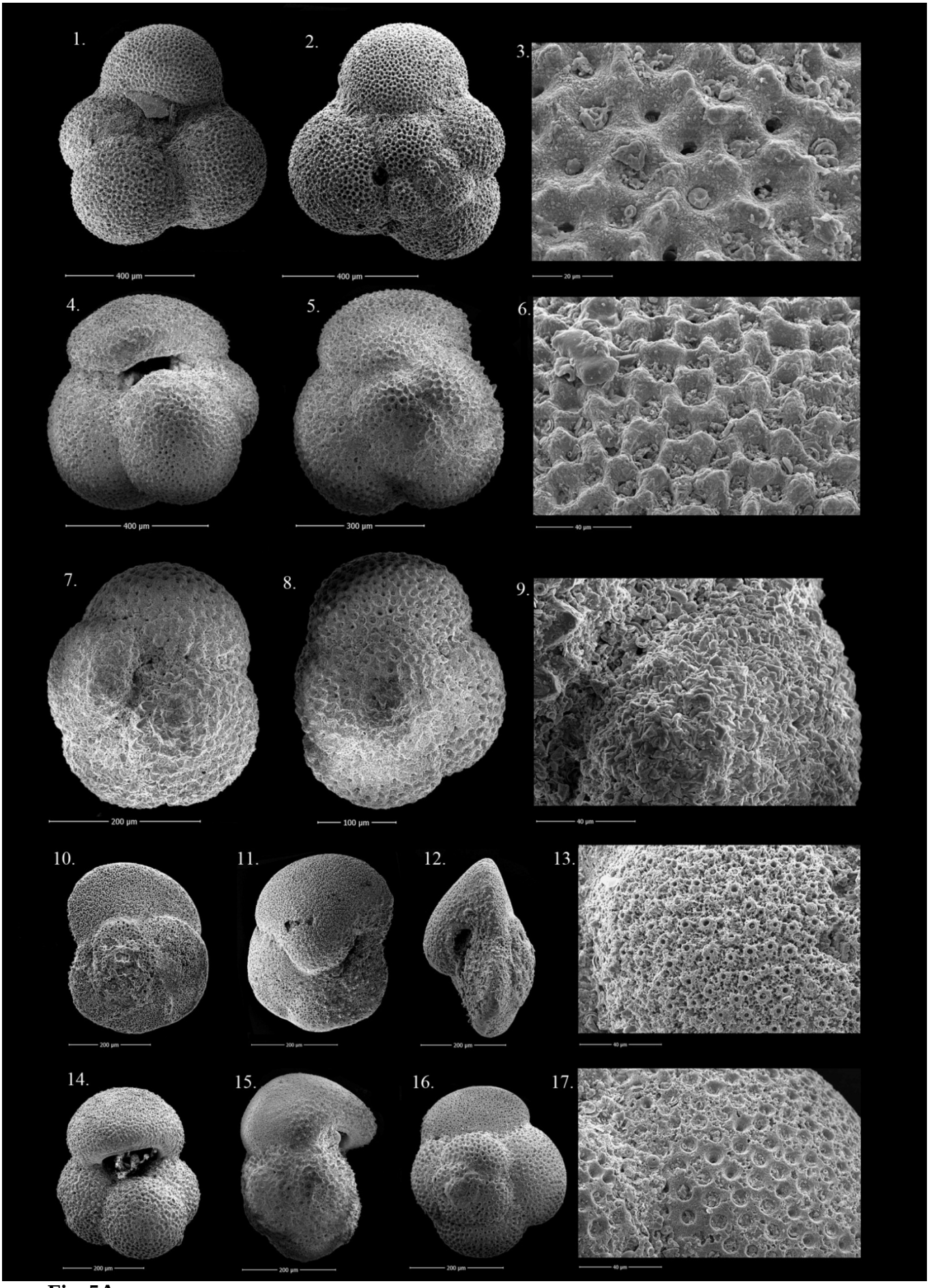

1.
2.
3.
400 µm
400 µm
20 µm
4.
5.
6.
400 µm
300 µm
40 µm
7.
8.
9.
200 µm
100 µm
40 µm
10.
11.
12.
13.
200 µm
200 µm
200 µm
40 µm
14.
15.
16.
17.
200 µm
200 µm
200 µm
40 µm

Fig. 5A

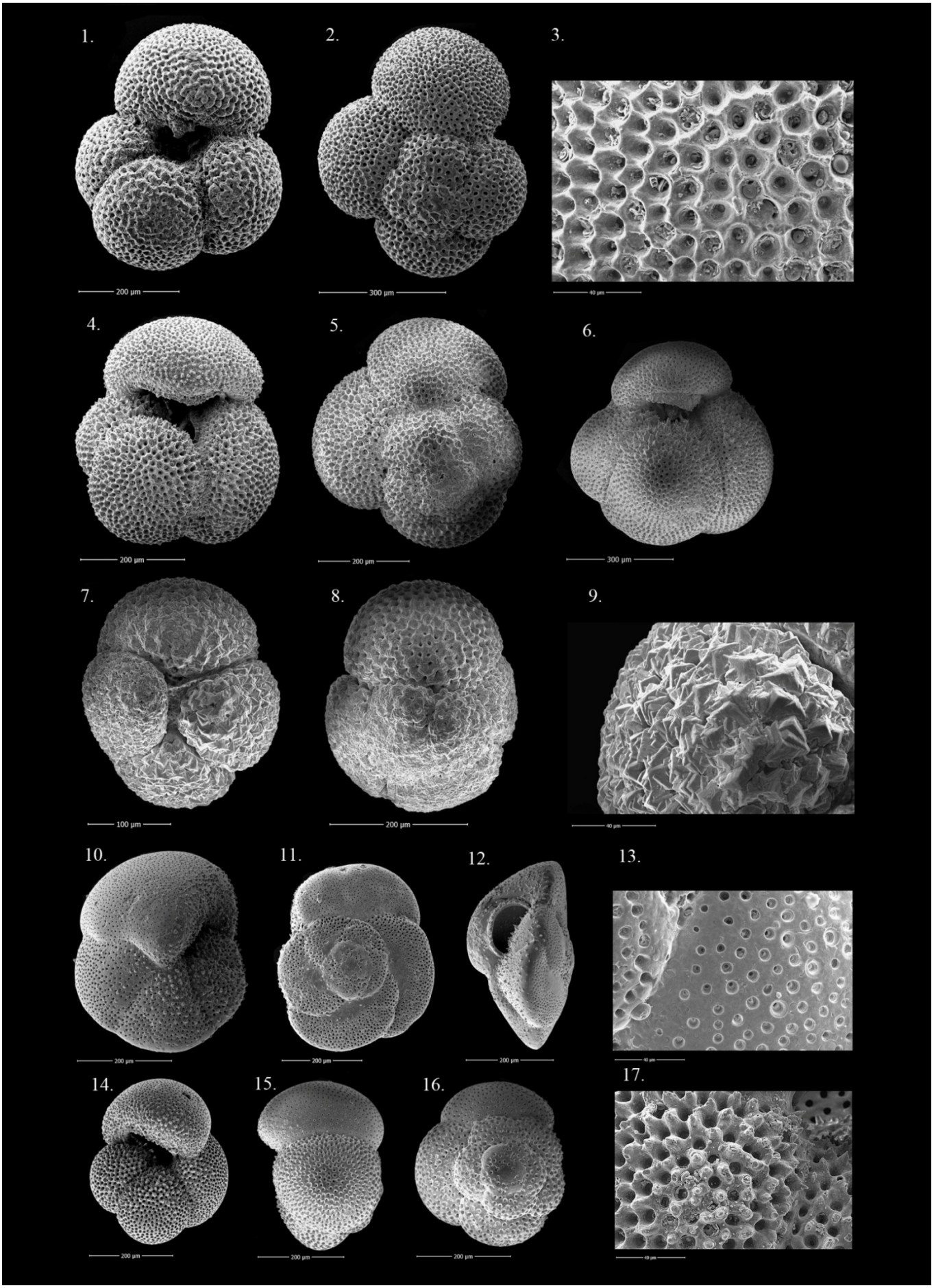

1.
2.
3.
200 µm
300 µm
40 µm
4.
5.
6.
200 µm
200 µm
300 µm
7.
8.
9.
100 µm
200 µm
40 µm
10.
11.
12.
13.
200 µm
200 µm
200 µm
40 µm
14.
15.
16.
17.
200 µm
200 µm
200 µm
40 µm

Fig. 5B

Fig. 5
SEM images representing specimen from late Eocene. Fig. 5A comes from ODP Site 1053B, and Fig. 5B represents IODP Site U1411B. 5A and & 5B from top, left to right: 1*) S. projecta*, umbilical view and 2) spiral view. 4) *D. venezuelana*, umbilical view (same species and view in image 6 from Fig. 5B) and 5) spiral view. 7) *C. unicavus*, umbilical view and 8) spiral view. 10) *T. cocoaensis*, umbilical view, 11) spiral view and 12) edge view. 14) *T. ampliapertura/ T. increbescens* umbilical view, 15) edge view and 16) spiral view. In Fig. 5A, 3, 6, 9, 13 and 17 represents the wall texture from each specimen from Site 1053B. Note the damage of secondary alteration in the wall texture compare to the nice surface wall texture in the images 3, 9, 13 and 17 from Site U1411B in Fig. 5B.

Isotopic analysis

As a preparation before the isotopic analysis all the species picked out from different fractions in different cores need to be weighed in and put in 12 ml vials. Each sample should weigh around 0,2 mg for getting the best result. The absolute minimum weight of a sample is 0,05 mg. Around this weight the standard deviation from the isotopic results may be a little higher because of the small amount of sample. Because of the low contents in some species in some fraction this low weight has been achieved in a few samples. 155 samples have been weighed for the isotopic analysis.
The goal with the isotopic analysis is to measure obtain $\delta^{13}C$ and $\delta^{18}O$ on the ecology of some late Eocene planktonic foraminifera and hopefully get values related to photosymbiotic evidence in the selected foraminifera.
The analysis has been made at Stockholm University using Gasbench II termo scientific instrument coupled to a masspectrometer MAT 252 termo scientific.
The samples has been dried at 50° in an oven over a night and then washed with He so the remaining air will disappear. The remaining Oxygen in the vials reacts with the foraminifera on the bottom of the vial. To get a reference, standard gases are also prepared for analysis. The standard gas (powdered carbonates from IAEA) is similar to the carbonates in the samples and is used to calibrate the reference gas CO_2.

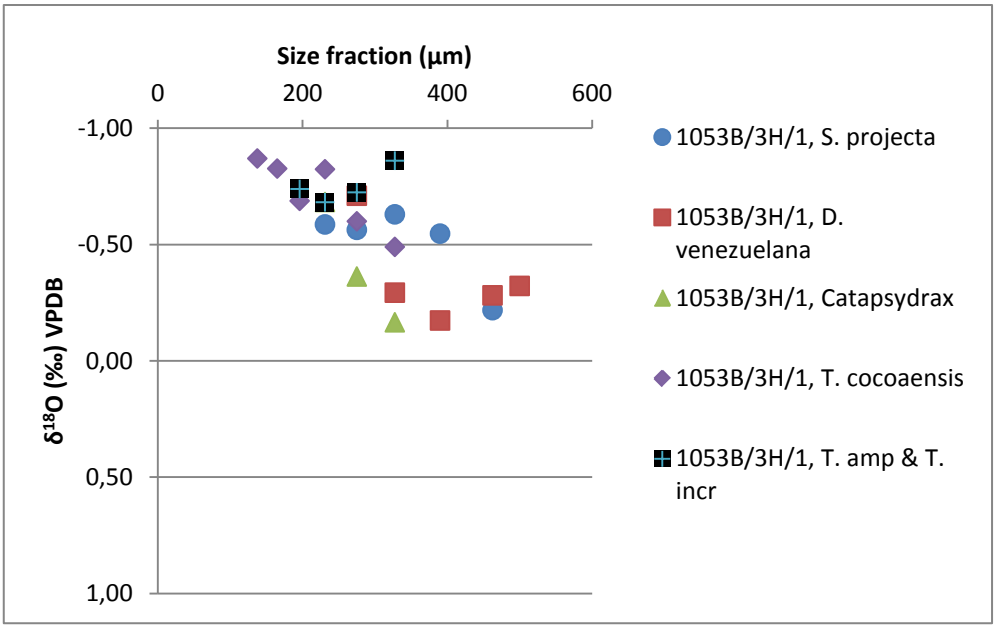
The analyzing moment

He passes through the sample and picks up the reference gas CO_2 on the way out. The CO_2 goes through the Gasbench and separates the gases from sample. Then the gases pass through the masspectrometer for analysis. Further calibration and diagrams will be presented in the result. The analytical precession for the isotopic instrument is 0,06 ‰ for carbon isotope and 0,15 ‰ for the oxygen isotope.

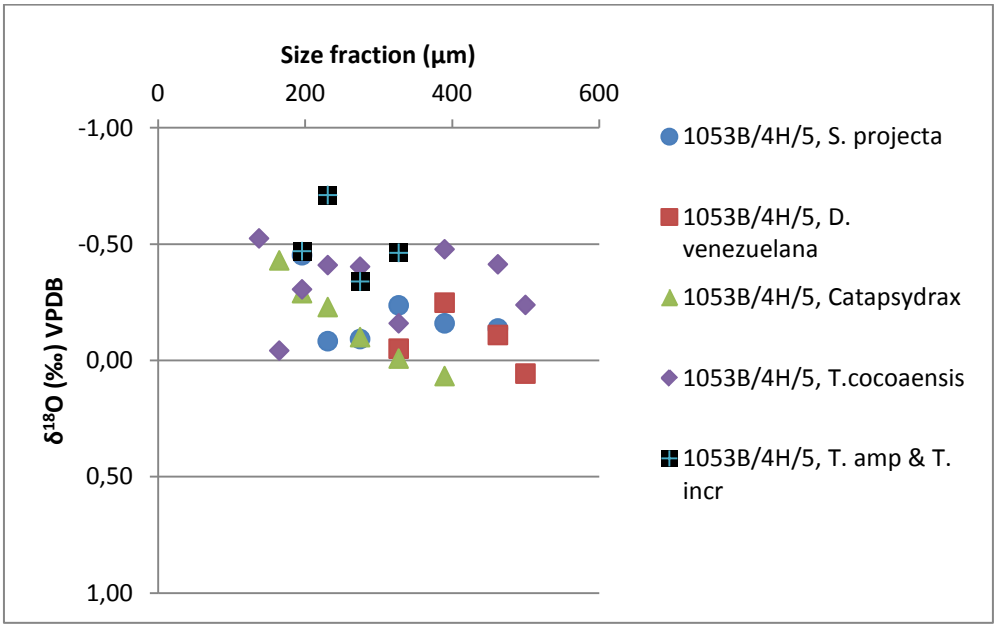
Results

The images from SEM analysis show that the specimens from U1411B exhibit a very good wall texture preservation and quality. **Fig**. **5B** in picture: 3,9, 13 and 17 have a clear wall texture with no alteration or recrystallization damages. In **Fig. 5A**, picture 3, 6, 9, 13 and 17 from site 1053B exhibit a more degraded wall texture which has undergone a conversion. The crystals have a more frosty texture and have been exposed for recrystallization (Sexton et al. 2006).

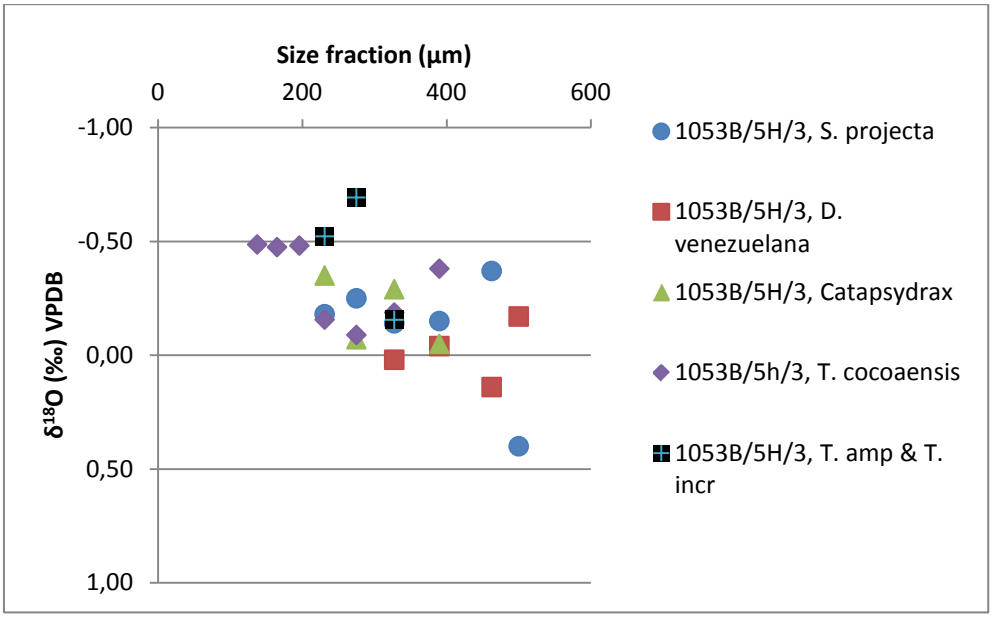
A.


Size fraction (µm)
0
200
400
600
-1,00
-0,50
0,00
0,50
1,00
δ18O (‰) VPDB
1053B/3H/1, S. projecta
1053B/3H/1, D. venezuelana
1053B/3H/1, Catapsydrax
1053B/3H/1, T. cocoaensis
1053B/3H/1, T. amp & T. incr

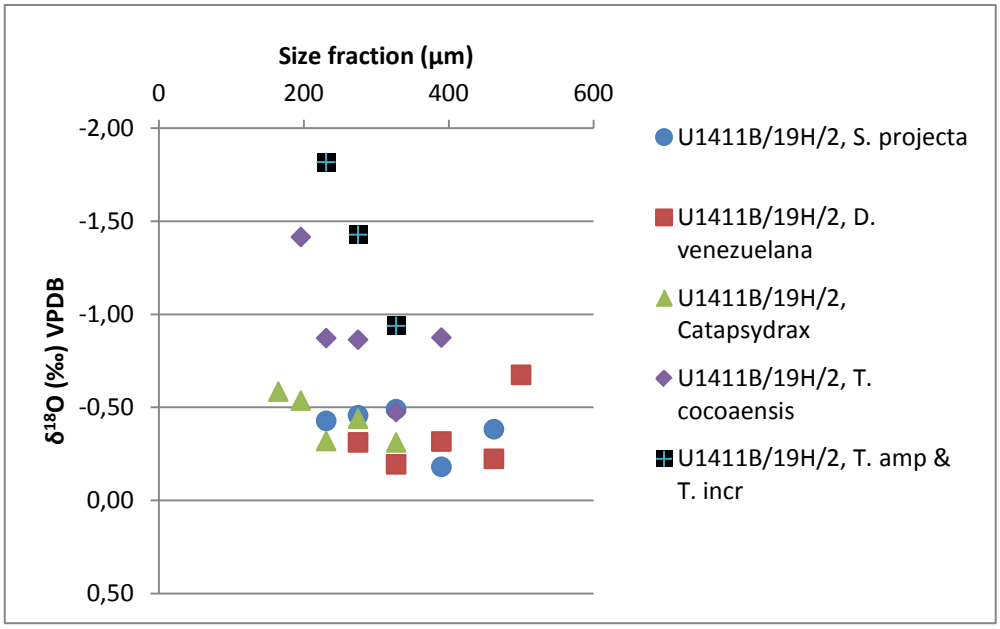
B.


Size fraction (µm)
0
200
400
600
-1,00
-0,50
0,00
0,50
1,00
δ18O (‰) VPDB
1053B/4H/5, S. projecta
1053B/4H/5, D. venezuelana
1053B/4H/5, Catapsydrax
1053B/4H/5, T.cocoaensis
1053B/4H/5, T. amp & T. incr

C.


Size fraction (µm)
0
200
400
600
-1,00
-0,50
0,00
0,50
1,00
δ18O (‰) VPDB
1053B/5H/3, S. projecta
1053B/5H/3, D. venezuelana
1053B/5H/3, Catapsydrax
1053B/5h/3, T. cocoaensis
1053B/5H/3, T. amp & T. incr

D.


Size fraction (µm)
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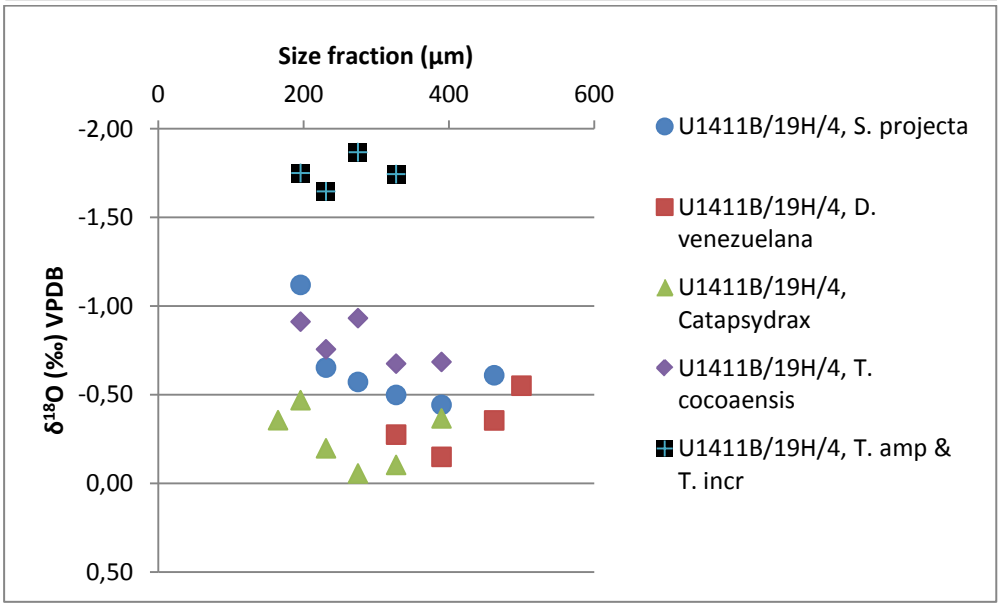
200
400
600
-2,00
-1,50
-1,00
-0,50
0,00
0,50
δ18O (‰) VPDB
U1411B/19H/2, S. projecta
U1411B/19H/2, D. venezuelana
U1411B/19H/2, Catapsydrax
U1411B/19H/2, T. cocoaensis
U1411B/19H/2, T. amp & T. incr

E.


Size fraction (µm)
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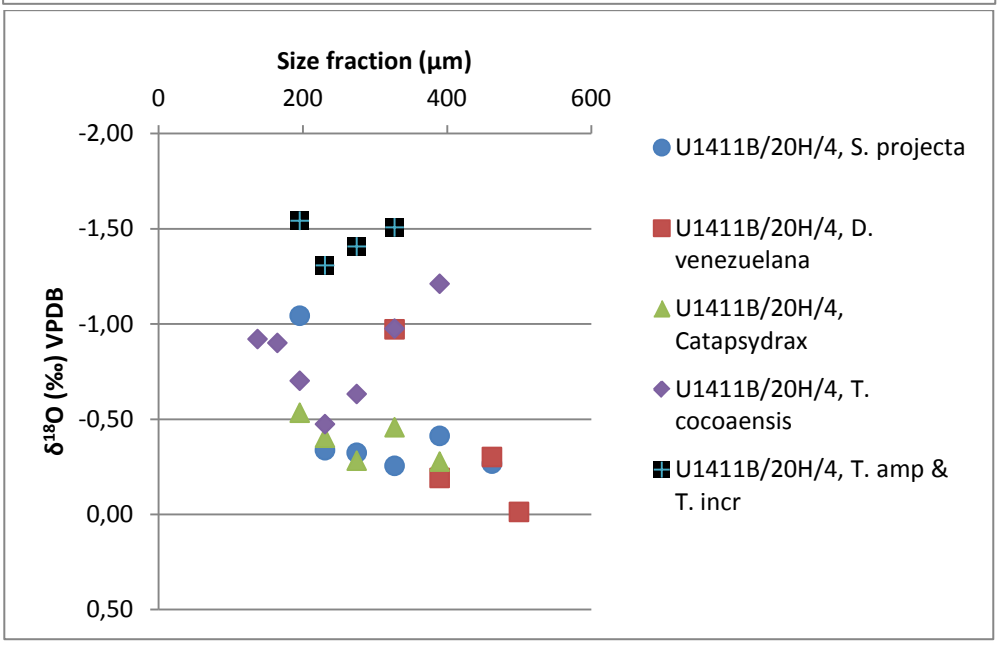
200
400
600
-2,00
-1,50
-1,00
-0,50
0,00
0,50
δ18O (‰) VPDB
U1411B/19H/4, S. projecta
U1411B/19H/4, D. venezuelana
U1411B/19H/4, Catapsydrax
U1411B/19H/4, T. cocoaensis
U1411B/19H/4, T. amp & T. incr

F.


Size fraction (µm)
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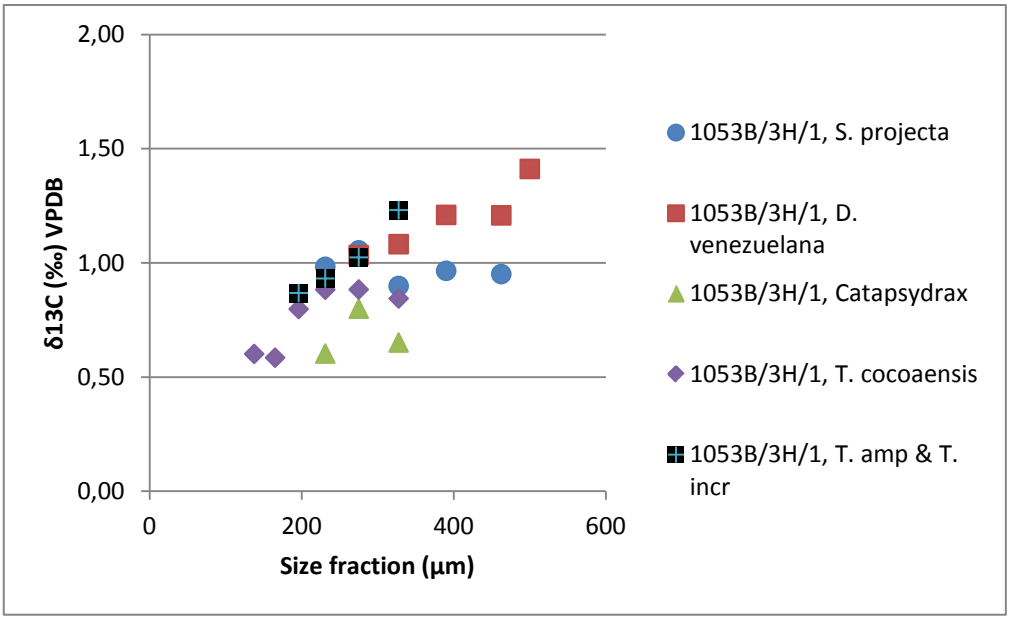
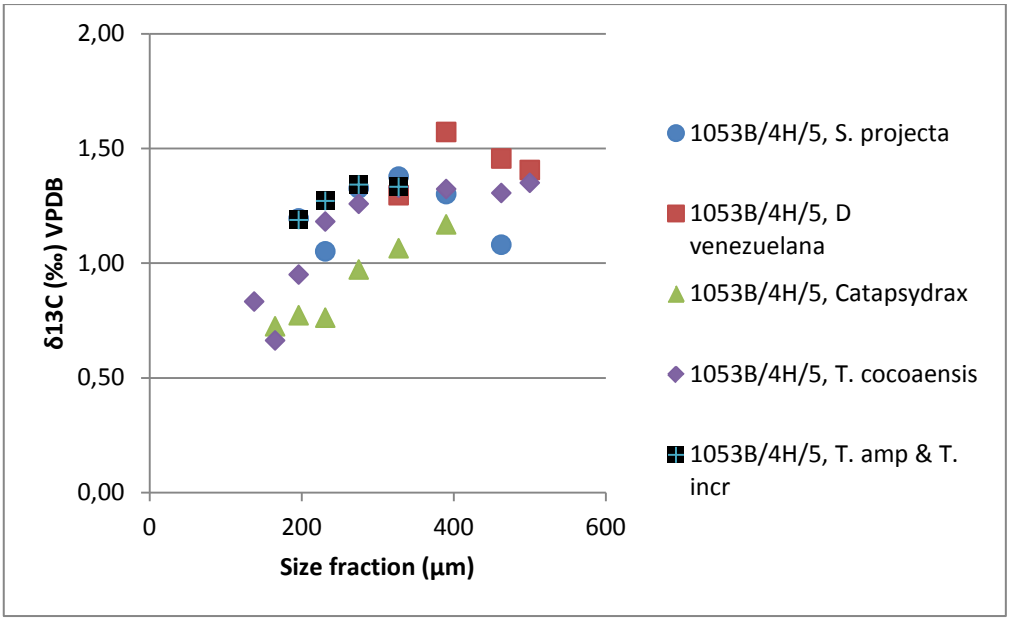
200
400
600
-2,00
-1,50
-1,00
-0,50
0,00
0,50
δ18O (‰) VPDB
U1411B/20H/4, S. projecta
U1411B/20H/4, D. venezuelana
U1411B/20H/4, Catapsydrax
U1411B/20H/4, T. cocoaensis
U1411B/20H/4, T. amp & T. incr

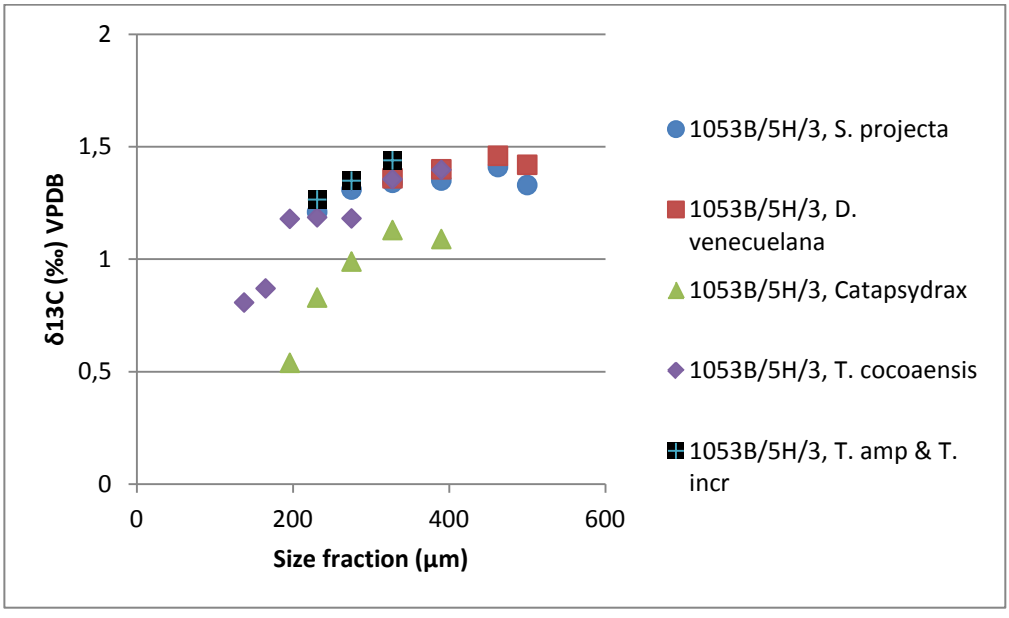
G.


2,00
1,50
1,00
0,50
0,00
δ13C (‰) VPDB
0
200
400
600
Size fraction (µm)
1053B/3H/1, S. projecta
1053B/3H/1, D. venezuelana
1053B/3H/1, Catapsydrax
1053B/3H/1, T. cocoaensis
1053B/3H/1, T. amp & T. incr

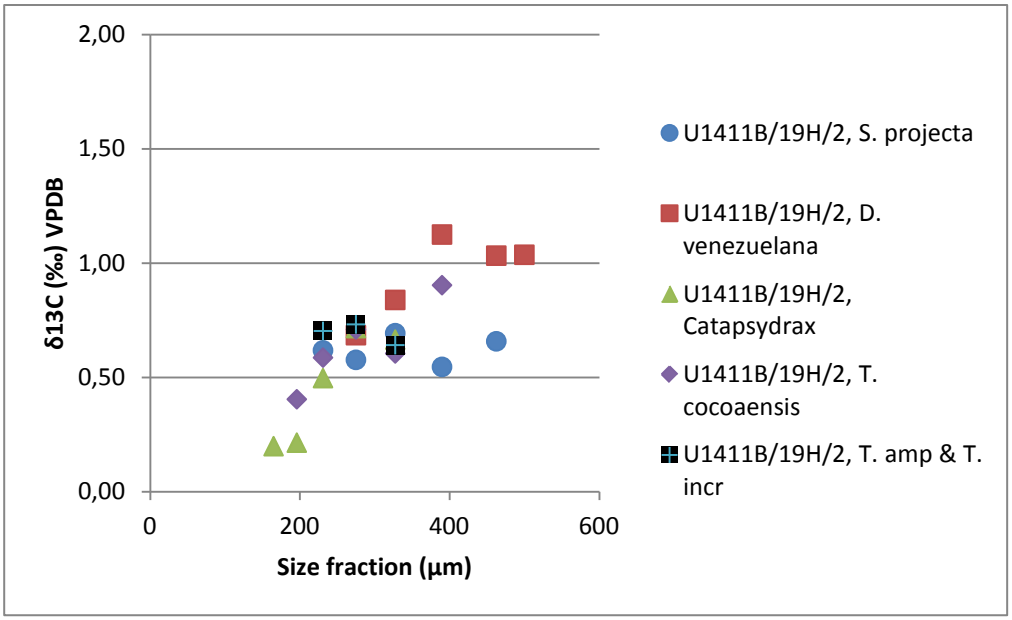
H.


2,00
1,50
1,00
0,50
0,00
δ13C (‰) VPDB
0
200
400
600
Size fraction (µm)
1053B/4H/5, S. projecta
1053B/4H/5, D venezuelana
1053B/4H/5, Catapsydrax
1053B/4H/5, T. cocoaensis
1053B/4H/5, T. amp & T. incr

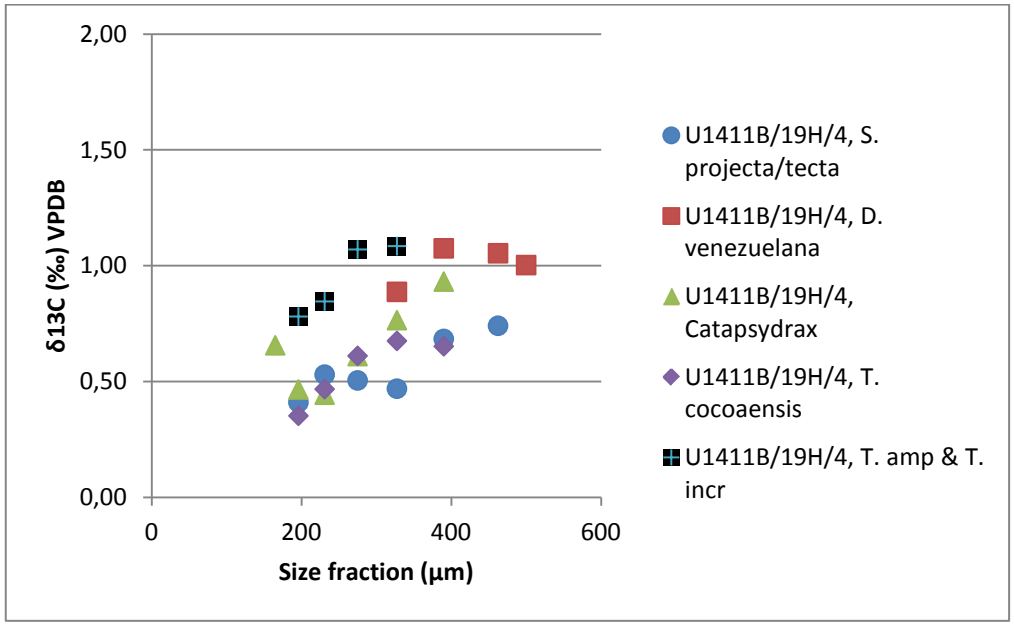
I.


2
1,5
1
0,5
0
δ13C (‰) VPDB
0
200
400
600
Size fraction (µm)
1053B/5H/3, S. projecta
1053B/5H/3, D. venecuelana
1053B/5H/3, Catapsydrax
1053B/5H/3, T. cocoaensis
1053B/5H/3, T. amp & T. incr

J.


2,00
1,50
1,00
0,50
0,00
δ13C (‰) VPDB
0
200
400
600
Size fraction (µm)
U1411B/19H/2, S. projecta
U1411B/19H/2, D. venezuelana
U1411B/19H/2, Catapsydrax
U1411B/19H/2, T. cocoaensis
U1411B/19H/2, T. amp & T. incr

K.


2,00
1,50
1,00
0,50
0,00
δ13C (‰) VPDB
0
200
400
600
Size fraction (µm)
U1411B/19H/4, S. projecta/tecta
U1411B/19H/4, D. venezuelana
U1411B/19H/4, Catapsydrax
U1411B/19H/4, T. cocoaensis
U1411B/19H/4, T. amp & T. incr

L.

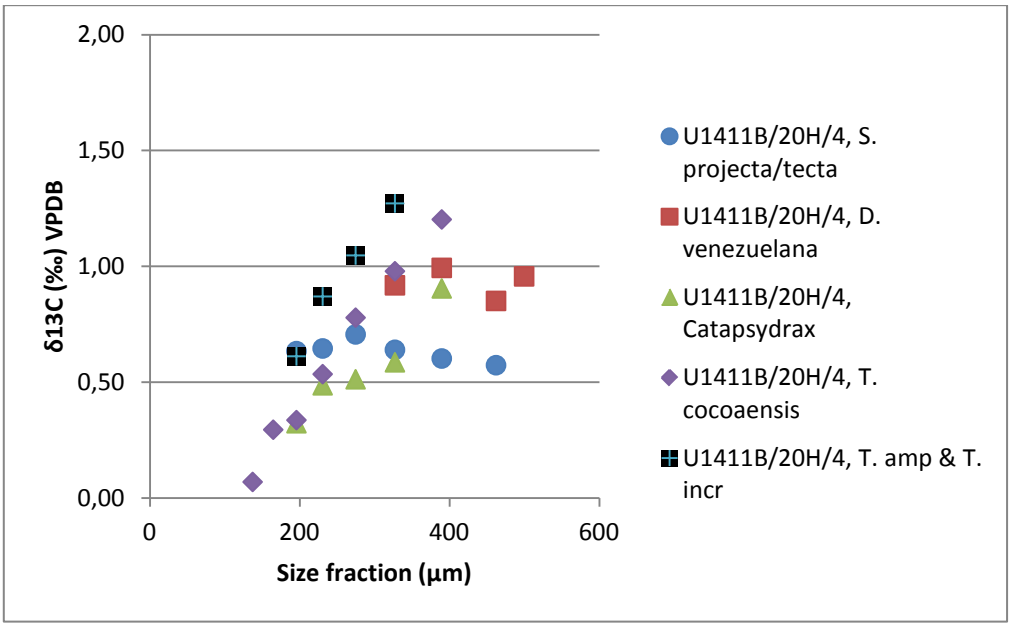

2,00
1,50
1,00
0,50
0,00
δ13C (‰) VPDB
0
200
400
600
Size fraction (µm)
U1411B/20H/4, S. projecta/tecta
U1411B/20H/4, D. venezuelana
U1411B/20H/4, Catapsydrax
U1411B/20H/4, T. cocoaensis
U1411B/20H/4, T. amp & T. incr

Fig. 6
Result of the stable isotopic analysis on *S. projecta/ S. tecta, D. venezuelana, Catapsydrax, T. cocoaensis* and *T. amp & T. incr*. A-C represent $\delta^{18}O$ from site 1053B (Blake Nose), D-F show $\delta^{18}O$ from site U1411B (Newfoundland margin). G-I show the $\delta^{13}C$ isotope signals from site 1053B and the $\delta^{13}C$ isotopic signals from site U1411B are shown in J-L.

All the values from the isotopic analysis have been calculated in the PDB (Pee Dee Belemnite) standard to achieve the stable isotopic results of $\delta^{13}C$ and $\delta^{18}O$.

$$\delta = \left(\frac{R_{sample}/R_{standard}}{R_{standard}} - 1\right) * 1000$$

R= ratio of $^{12}C/^{13}C$ or $^{18}O/^{16/}O$.

Trends in $\delta^{18}O$ stable isotope signals.

The isotopic signals show a lot of variability in the diagram between species across different size fractions but also between the two sites.
In general site U1411B (range between 0 to -2 ‰) exhibit more negative values of the $\delta^{18}O$ and are also more spread than site 1053B (which exhibit a range between 0,5 to -1 ‰). The most interesting pattern is that *T. ampliapertura* and *T. increbescens* show the most negative values of $\delta^{18}O$ in **fig. 6,** A, E and F compared to the other species. *Catapsydrax, Subbotina, Dentoglobigerina* and some extent of *T. cocoaensis* shows the highest values of $\delta^{18}O$ and the values increases by size. There is a slightly down going trend to higher $\delta^{18}O$ values with increasing size, most of the species in both sites show this trend.

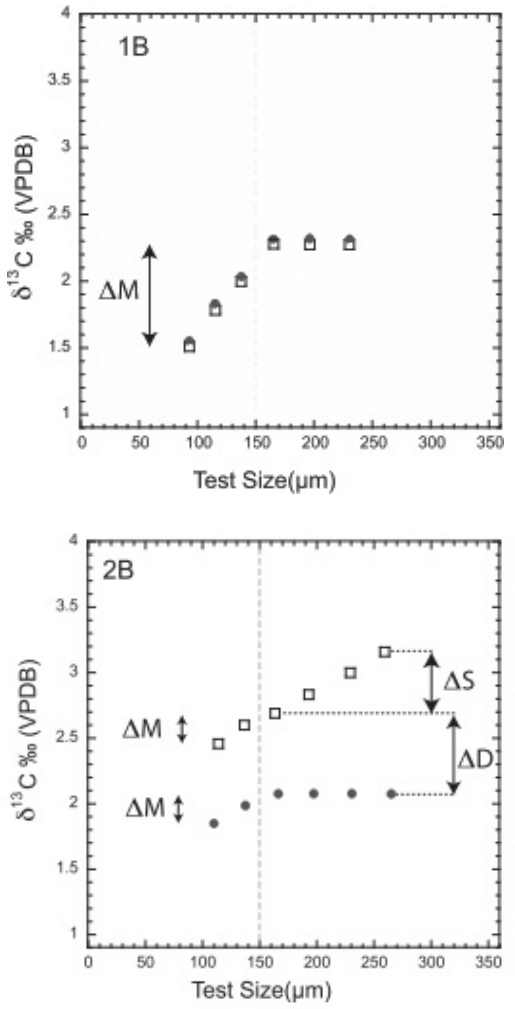
Trends in $\delta^{13}C$ stable isotope signals.

General for all the diagrams is that $\delta^{13}C$ is showing a positive increase with increasing size.
The diagrams from each site all show differences in the pattern, but have a positive general slope in between ~0 ‰ - 1,5‰. In site U1411B the $\delta^{13}C$ are displaced by somewhat lower isotopic signals (range between 0 to ~1 ‰) compare to site 1053B (range between 0,5 to ~1,5 ‰). *Catapsydrax, T. cocoaensis* and *T. ampliapertura/T. increbescens* have a steeper gradient of the $\delta^{13}C$ with increasing size compare to the other species. This is seen in both of the sites. *Subbotina* , at Site U1411 tends to show a little increase in $\delta^{13}C$ at test sizes above 250 µm (**fig. 6,** J and K) in the diagram. **Fig**. **6**, I from site 1053B show a similar gradient. *D. venezuelana* shows a similar pattern except in the smaller fractions. The steepest gradient of $\delta^{13}C$ is shown in Fig. F from U1411B/20H/4. This sample had the greatest enrichment in planktonic foraminifera species and also exhibited the best preservation.

Discussion

Birch et al. 2012 show $\delta^{13}C$ and $\delta^{18}O$ trends in evolving species from the early Paleocene. From their results they interpreted two biological effects in the size related $\delta^{13}C$ gradients of the planktonic foraminifera species studied. Their results show gradients for a metabolic effect in the smaller size fractions (<200 µm), and photosymbiosis in the higher size (>250 µm). These effects of photosymbiosis versus metabolic effect are illustrated below in a hypothetical model (**fig. 7**) (Birch et al. 2012).

A.



B.

Fig. 7.

A, shows the hypothetical metabolic pattern (ΔM) of a $\delta^{13}C$ stable isotope sequence for one individual microorganism. B, shows the symbiotic pattern for one individual microorganism. This picture is taken from: Evolutionary ecology of early planktonic foraminifera: size, depth habitat and photosymbiosis (Birch et al. 2013)**.**

In another study by Paul Pearson and Bridget Wade based on paleoecology in planktonic foraminifera from late Oligocene, collected from warm tropical water also show this similar pattern of metabolic effect and photosymbiosis. Shown from the $\delta^{13}C$ and $\delta^{18}O$ stable isotope signals in the water column. They have collected all their studied species and simulated them in a water column based on the stable isotopic result (**Fig. 8)**

The relationship between δ18O and water temperature provides indications of the relative depth position that different planktonic foraminifera species lived at in the water column during early Oligocene. (Pearson & Wade, 2009).

The purpose of this is to see if the planktonic foraminifera show pattern of warmer mixed layer condition or more sub thermocline to deeper thermocline condition. More negative values of $\delta^{18}O$ indicate a warmer habitat in the mixed layer whereas higher values indicate a colder and deeper habitat in the thermocline or sub thermocline (Birch et al. 2013). *Subbotina, Dentoglobigerina* and *Catapsydrax* exhibiting higher values of $\delta^{18}O$ and were probably living deeper down the water column. This is general for both of the cores.

However small differences of higher $\delta^{18}O$ signals due to increasing size, may indicate migration of a species down the water column with adding of more calcite during gametogenesis (Pearson et al. 2001), In this study, *Catapsydrax* and *T.cocoaensis* are exhibit this pattern.

A Planktonic foraminifera species that exhibit photosymbiotic pattern need to live high in the photic zone (down to approximately 200 m below sea surface) where all the photosynthetic activity occur and the sun irradiance is high (Birch et al. 2013). According to the $\delta^{18}O$ stable isotope signals in this study, *T. ampliapertura* and *T. increbescens* exhibit these characteristics while *T. cocoaensis* and *Catapsydrax* show a more migration pattern with increasing size.

The diagram D-F exhibit $\delta^{18}O$ isotopic values that comes from site U1411B which contained very well preserved

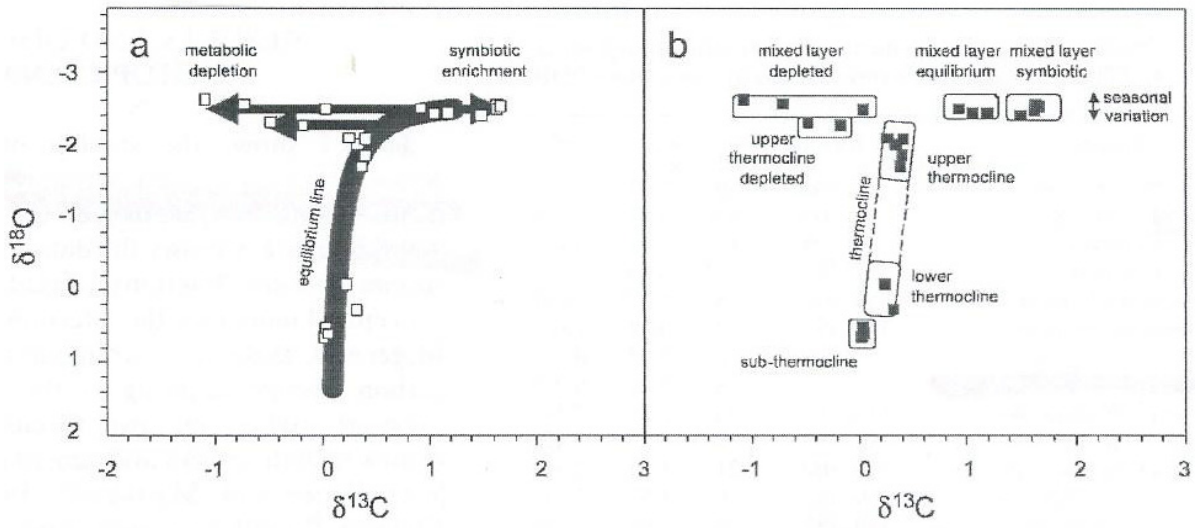


Fig. 8.
This image simulates the water column structure from a study of late Oligocene planktonic foraminifera assemblage, based on the stable isotopic signals. Combining $\delta^{13}C$ and $\delta^{18}O$ results show the metabolic effect and photosymbiotic pattern for different species. Picture is taken from: Taxonomy and stable isotope paleoecology of well-preserved planktonic foraminifera from uppermost Oligocene of Trinidad (Pearson & Wade, 2009).

foraminifera, with no visible alteration or recrystallization pattern compared to **fig. 6,** A-C from site 1053B which exhibit pore preservation with recrystallization and considerable infillings of apertures (see **fig. 5).**
This is the result of the clay rich sediment that site U1411B possesses, where pore water has not interacted with the calcite in foraminifera, so their shell exhibit a better preservation (Sexton et al. 2006).
According to previous studies on recrystallization in planktonic foraminifera shell structure from the late Cretaceous and Eocene show that foraminifera that exhibit bad preservation in terms of alteration or recrystalization pattern show higher values in the $\delta^{18}O$ signals while the well preserved microfossil have a more negative value in the signals (Pearson et al. 2001).

In this study site U1411B shows more negative values in the $\delta^{18}O$ and a bigger spread between species compared to site 1053B. This may be due to high quality of preservation in U1411B and recrystallization of the planktonic foraminifera shell structure in Site 1053B.
Previous study of *T. cerroazualensis* which is related to *T. cocoaensis* gave relatively high $\delta^{18}O$ indicating a thermocline habitat. Also it did not show any $\delta^{13}O$ size related pattern of photosymbiotic activity, but exhibit smaller change of $\delta^{13}C$ inclination in the smaller size. *T. cocoaensis* show similar pattern (Wade et al. 2008 and Wade & Pearson, 2008). This may be due to a metabolic effect during respiration and not symbiotic activity.

The trend signals in the $\delta^{13}C$ stable isotopic result **fig. 6. G-L** shows a positive increase with increasing size.
These trend signals can depend on including (1) kinetic fractionation during the calcification process, (2) respiratory of CO_2 (metabolic effect) that utilized in the calcification of the shell and (3) photosymbiotic activity (D´Hondt et al. 1994).
To understand what the increase in carbon depend on it is important to interpret the

$\delta^{18}O$ and combined these trends with the trends of $\delta^{13}C$ signals for each single species.
D. venezuelana and *S. projecta/ S. tecta* has a low increasing gradient (more distinctive in *D.venezuelana*) in **fig. 6. G-L** and show also a less negative $\delta^{18}O$ signal. These data indicate a smaller fractionation ore metabolic effect in the smaller size and a deeper living habitat. This does not match the photosymbiotic characteristics.
General in **fig. 6. G-L** *Catapsydrax* and *T. cocoaensis* show a steeper $\delta^{13}C$ increasing gradient than *D. venezuelana* and *Subbotina*. This pattern reveals more fixed carbon in there shell, which are closer to photosymbiotic characteristics. But the $\delta^{18}O$ for these specimens show an increase with increasing site. This could be due to a migration down the water column during their life cycle.
An interesting finding in this study comes from stable isotopic signals in *T. ampliapertura/ T. increbescens.* The $\delta^{18}O$ signals indicate that this group were living in the upper surface mixed layer (in photic zone), because of the negative values compared to the other species. But they are also showing an positive $\delta^{13}C$ enrichment gradient with increasing size even above 250 µm (although sample numbers are small), as well as the most positive $\delta^{13}C$ values overall which may correspond to a small photosymbiotic pattern and not a metabolic pattern.
Carbon is more stable than $\delta^{18}O$ even in the recrystal material (not so spread out) with the same cores from the $\delta^{13}C$ signals where the same species show a positive increase with increasing size.

Higher values of $\delta^{13}C$ in site 1053B can depend on local enrichment in nutrients supply and surface water mixing (Birch et al. 2012).

Conclusion

High light intensity (representing surface mixed layer water) in planctonic foraminifera with $\delta^{13}C$ enrichment demonstrate photosymbiotic activity and give a positive offset of $\delta^{13}C$ and are not in equilibrium with the ambient DIC in ocean. This isotopic vital effect is essential knowledge for a paleoclimatologist studying the marine biological pump which links to the paleoclimate. This can be useful to understand changes in climate and climate development in the future.
The results presented here in this project suggest that a photosymbiotic pattern exists in the late Eocene taxa *T. ampliapertura* and *T. increbescens*. But further studies are needed from other cores with better preservation. It is also most important to include a wider interval of size fraction to be certain about the symbiotic existence in the species. Larger size interval (>150 µm) may show a longer steeper positive $\delta^{13}C$ slope and with high negative $\delta^{18}O$ indicating of shallow surface habitat. This will give more weight on the photosymbiotic hypothesis of the *Turborotalia ampliapertura/ T. increbescens* and maybe some other species that have not yet been studied.
Uncertainties in the samples can depend on the approaching to a colder climate change in the Eocene-Oligocene climate transition with a sea temperature drop and the formation of Antarctic ice sheet, resulting in disturbances in the ecology. This study represent a small building block for further studies about planktonic ecologies and changed habitat during global climate change for recreation of paleoclimate.

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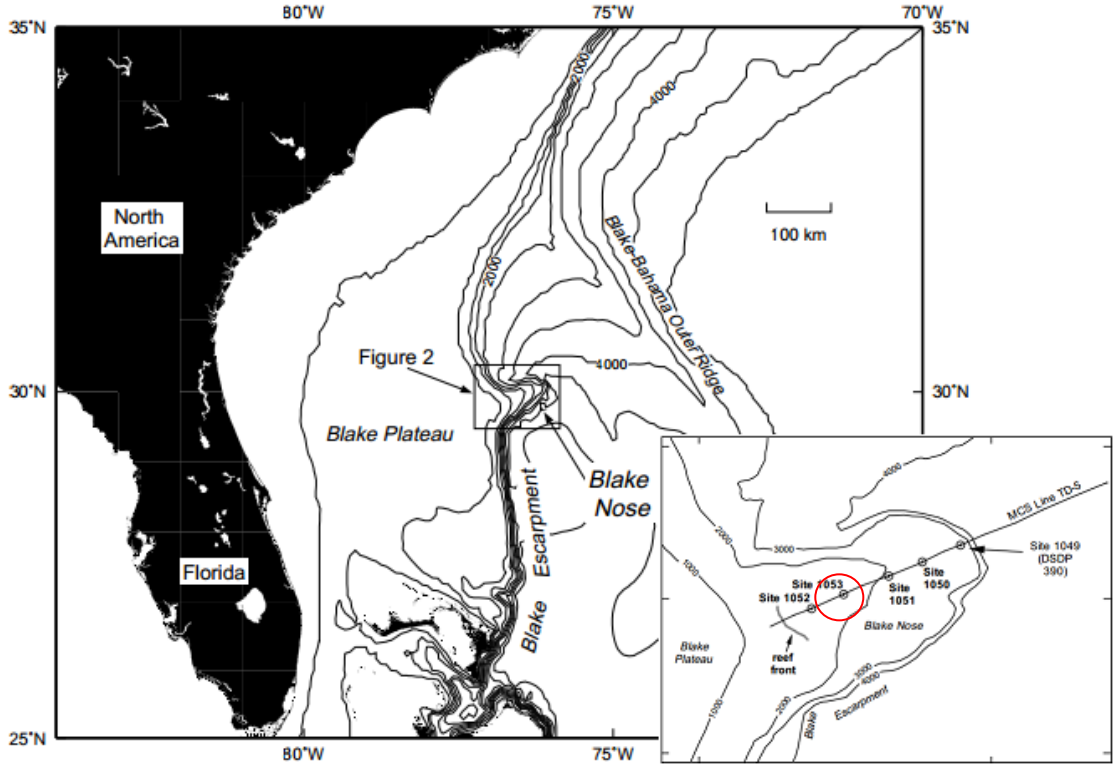
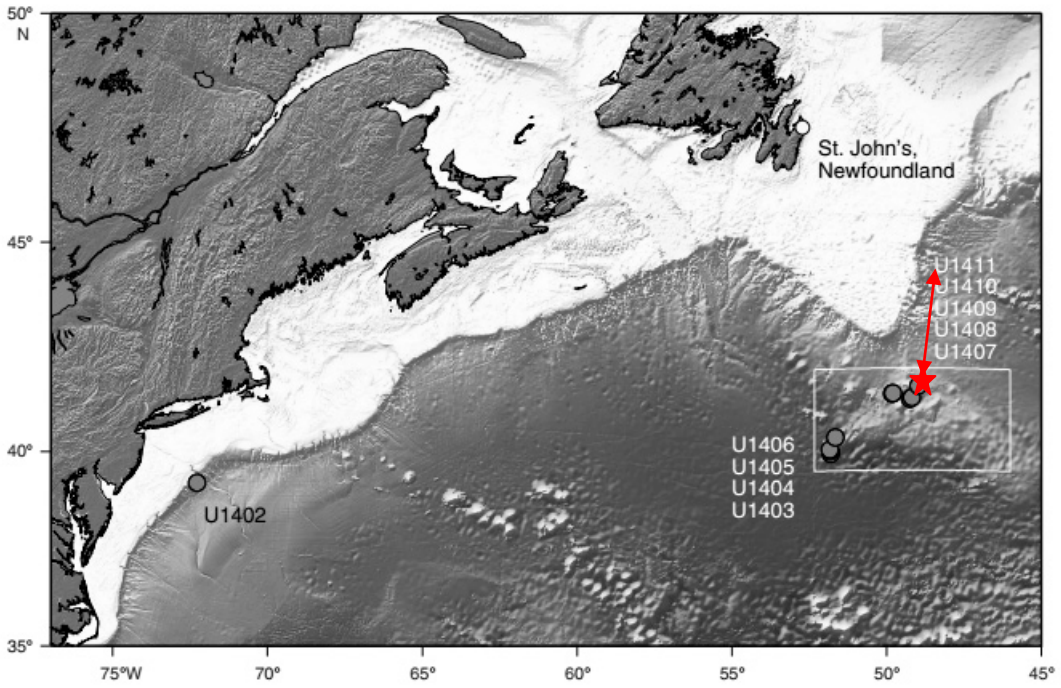
Appendix 1





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