Methods

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Analyses of planktic foraminifers are targeted towards three main goals. (1) The understanding of biological prerequisites and ecological demands of modern species facilitated by collection of planktic foraminifers at sea, and culturing of specimens in laboratory experiments. Those samples and associated data provide means for the analyses of the temporal and regional distribution, including depth habitat, availability of food, temperature, salinity, a variety of chemical parameters (i.e., stable isotopes, element ratios, pH, and other parameters of the marine carbonate system), and the availability of light (i.e. quality and intensity) to the symbiotic algae of foraminifers. (2) Proxy calibration and application of foraminifers in biostratigraphy, paleoecology, paleoceanography, and paleoclimate reconstruction (see Fischer and Wefer 1999, and reference therein). In addition to analyzing population dynamics and faunal assemblages of planktic foraminifers, (3) technology for biogeochemical analyses of foraminifers has been developed since the early 1950s, and is still being improved and extended to define and calibrate new proxies on the foraminifer shell, as well as on population dynamics (e.g., transfer functions). In this chapter, those methods are presented and discussed, which are most applied in sampling and analyses of planktic foraminifers at sea and in the laboratory. Given the rapid development of analytical methods, we provide merely an introduction to the various methods applied today, meant as first step to find the latest information published in specialist journals.

10.1 Sampling

While sampling planktic foraminifers from the water column, and processing of samples in the laboratory, any alteration of the individuals, tests, and assemblages is to be avoided (in theory), or kept at a minimum. When storing and conserving samples obtained from the water column, care should be taken to avoid fragmentation and dissolution of the planktic foraminifer tests, and to keep the pH of processing and storing liquids ≥ 8.2 at all times. In particular, processed fresh water from shipboard tanks, and deionized waters from laboratory devices may be delivered at low pH. pH should hence be monitored to avoid irreparable damage to samples.

Assemblages can not be entirely sampled with plankton nets, and specimens smaller than the net gauze will not be quantitatively included in the samples. When sampling with sediment traps, trapping efficiency, which usually deviates from 100 %, impedes complete samples. In turn, dissolution of tests or precipitation of any substance from the sampling solution on top of the foraminifer test can be avoided through correct and careful treatment of samples. Whereas some sampling artefacts may be overlooked and become clear only during data analyses, inadequate sampling of live individuals for culturing experiments emerges at once through inactivity or death of individuals. Proper sampling and processing hence constitutes the basis of any scientific work, good results, and fun at work.

10.1.1 Manual Collection of Live Specimens by SCUBA Divers

Planktic foraminifer specimens for culturing are ideally being sampled by hand at 'blue water' locations. Wide-mouthed glass jars are used as sampling containers to guarantee for minimal disturbance of specimens, and to avoid damage of cytoplasm and test, and particularly of the fragile spines (Hemleben et al. 1989; Huber et al. 1996). Spinose species are relatively easy to detect with the naked eye due to their large diameter, and could be sampled from oligotrophic (blue) surface waters using standard SCUBA equipment and techniques. Foraminifer specimens are relatively easy to detect at a distance of 50–80 cm in sunlit waters, and against a dark background such as the hull of a ship. Opening the lid of the jar close to the specimen to be collected will suck the foraminifer into the jar. Glass jars of about 125 mL are large enough for later culturing of specimens in their original ambient seawater for the first days. During the transport to the culture laboratory, the jars should be kept at constant temperature in an insulated chest. Back at the laboratory, the specimens could be kept in the original jars, or may be transferred to other culture vessels. Ambient seawater should be collected together with the foraminifers to serve as replacement water, and treated in the same way (e.g., same temperature) as the culture vessels containing live specimens.

10.1.2 Assemblage Sampling

For assemblage analyses and biogeochemical analyses, planktic foraminifers are preferably sampled with plankton nets. Some of the seminal early studies of Bé and co-workers were carried out using nets with rather large mesh-sizes between 200 and 366 μm (e.g., Bé 1960; Tolderlund and Bé 1971; Bé and Hutson 1977). Those nets are comparatively inexpensive, robust, and allow quick hauls, but do not sufficiently capture small-sized species, as well as pre-adult specimens of most modern planktic foraminifer species. Attempts have been made to use small mesh-sizes between 30 and 80 μm (e.g., Schott 1935; Phleger 1945), and which need to be hauled very slowly (up to 0.3 ms^{-1}) to avoid tearing of the gauze. In addition, fine-meshed nets easily get clogged with particulate matter in mesotrophic and eutrophic waters, and back-pressure of the water eventually impedes quantitative sampling. 100-μm nets have been proven good compromise between employability onboard research vessels, and applicability to faunistic studies, although some of the small-sized species (e.g., tenuitellids) might still be largely underrepresented in the samples, in comparison to the original planktic foraminifer populations. The volume of sampled seawater is quantified with flow meters (see text box on Flow meters).

> **Flow meters:** Flow meters, analogous and digital, installed at the outside *and* inside of the plankton net and CPR, provide independent data on the volume of seawater sampled (e.g., Motoda et al. 1957). Flow meter data are particularly important to calibrate new sampling devices, and to measure volumes of sampled seawater under varying sampling conditions, such as different hauling speeds. Resulting calibration curves may later be applied to correct for sampling errors, which are most certain to occur when working at sea under sometimes unforeseen and difficult conditions. In addition, winches of vessels, which are poorly equipped for scientific sampling (like some 'ships of opportunity'), might not be manufactured for precise adjustment of hauling speeds. Resulting deviations in sampled seawater volumes may later be corrected by using flow meter data. Even winches of research vessels may turn out to be less adjustable than expected.

10.1.3 Single Nets

The smallest, lightest, and cheapest option for sampling planktic foraminifers from the upper water column is the Apstein net, optionally being

hauled by hand and employable even from small zodiacs. Apstein nets of typically 25 cm diameter and 50 or 100 cm length are suited to sample well preserved specimens for culturing and geochemical analyses, but are not suited for quantitative sampling of the planktic foraminifer fauna. Larger ring-nets (e.g., 'bongo nets') may be employed for quantitative sampling, and require a vessel with an adequate winch for scientific sampling.

10.1.4 Multiple Opening-Closing Nets

Multiple opening-closing nets (MCNs) have been employed for vertical and horizontal tows of up to nine sampling intervals, depending on the design of the device (Bé et al. 1959; Bé 1962; Wiebe et al. 1976). Apertures of the multi-nets usually range between 0.125 and 1 m^2 (see, e.g., www.hydrobios.de). Multi-nets with 0.25 m^2 (50 cm × 50 cm) opening and five net bags have proven most suitable for sampling of planktic foraminifers, and to be deployed from ships of different size and equipped with different types of winches (Fig. 10.1). MCNs are large and heavy enough to be employed with long and (optionally, for manual release) conductive wire down to a maximum water depth of 3000 m (recommendation of the manufacturer), and may be hauled at a speed of 0.5 ms^{-1} for quantitative sampling of the water column when using 100-μm gauze.

Fig. 10.1 Multinet equipped with five 100-μm nets (type Hydrobios midi, 50 × 50 cm opening) returning from vertical haul on the French research vessel 'Marion Dufresne' in the southern Indian Ocean. The sampling cups (*red*) are placed in a rack below the plankton-nets. An addition weight below the cup-rack keeps system straight. *Photo* H. Howa, Angers University, France, with permission
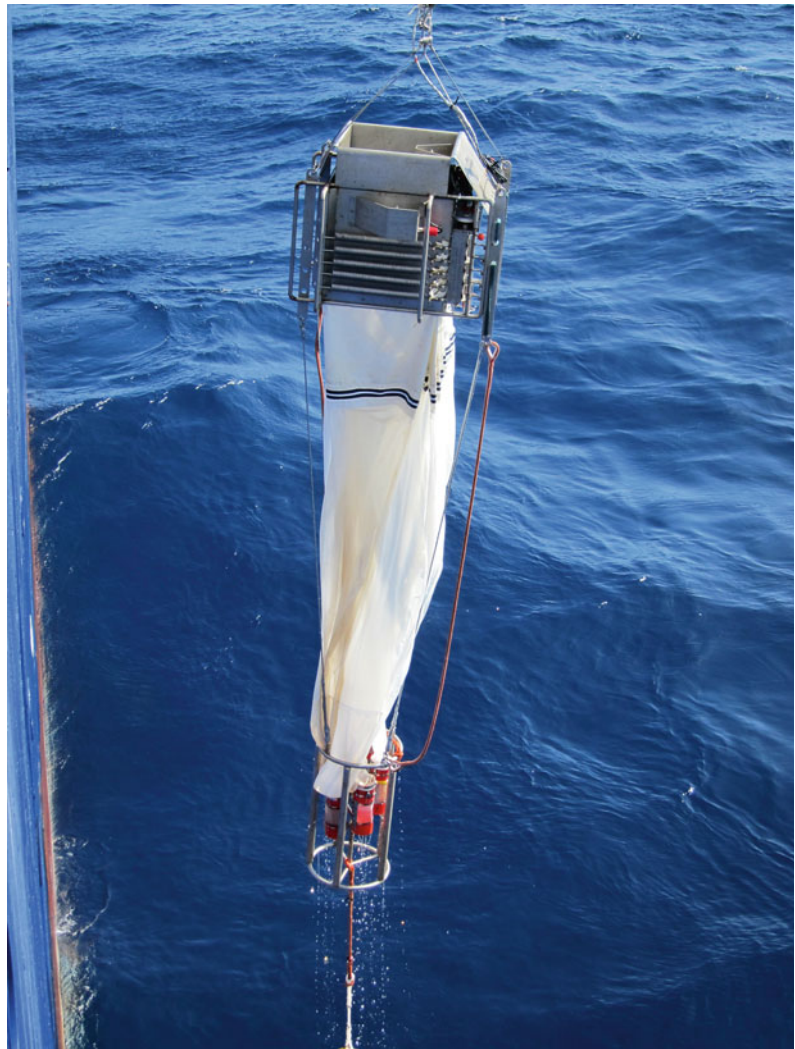
New makes of nets can be employed off-line, and conductive wire is hence not imperative. Hauling speeds are adjusted to the size of net-gauze used with the MCN, and need to be adapted to weather conditions. Rough sea and rolling ship requires low hauling speeds (e.g., 0.3 ms^{-1}) to prevent tearing of the net gauze at sharp increases of back-pressure of the sampled water, which may be caused by rapid movement of the ship. Samples obtained under different from 'normal' conditions may not be directly comparable with samples obtained with other hauling speeds. If no Apstein net or MCN is available, any other type of net could be used for sampling such as, for example, a larger MOCNESS (please be aware of idiosyncratic terminology of net types).

10.1.5   Continuous Plankton Recorder (CPR)

A Hardy-Plankton Recorder (also Longhurst-Hardy Plankton-Recorder, LHPR; or Continuous Plankton Recorder, CPR) is towed through the surface water column behind a sailing ship at different water depths, and produces under-way samples for quantitative analyses (e.g., Hardy 1935; Longhurst et al. 1966; Reid et al. 2003; Sir Alister Hardy Foundation for Ocean Science, http://www.sahfos.ac.uk/). To avoid tearing of the sampling gauze (called silk) by back-pressure of the sampled waters, rather coarse mesh-sizes ($\geq 200 \text{ }\mu\text{m}$) have usually been used. Pre-adult and small-sized planktic foraminifers may hence not be quantitatively sampled, and a large part of the fauna might is missed.

10.1.6   Seawater Pumps

An elegant method of sampling planktic foraminifers from surface waters is the use of shipboard seawater-pumps, which can be employed during sailing, and hence consume no additional (i.e. costly) on-site ship-time. For example, deck wash pumps and fire pumps may be employed, provided that the sampled water is neither contaminated nor compressed while being pumped, which could cause damage to fragile specimens. Air bubbles produced along the hull of the sailing ship may affect the water intake, which needs to be taken into account to correctly assess the volume of the sampled seawater. The pumped seawater may be sampled with an Apstein net (cf. Ottens 1992).

10.1.7   Sampling the Test Flux

Continuous sampling of settling planktic foraminifer tests is carried out with moored or drifting sediment traps of varying design (cf. Buesseler et al. 2007). Automated sediment traps of conical shape have been used since the 1970s, usually derived from traps of the WHOI PARFLUX design (see Honjo et al. 1980 for the PARFLUX Mark II trap). Moored sediment traps typically have a 1-m^2 opening (size of the sampling area), and are equipped with 24 sampling cups. A baffle grid covers the opening to keep large swimmers and large 'particles' off the inside of the trap to avoid any damage of the sampled matter (e.g., Wiebe et al. 1976; Honjo and Manganini 1993; Lampitt et al. 2008, and references therein). Depending on region and water depth, current strengths, and lateral transport of tests (Siegel and Deuser 1997; von Gyldenfeldt et al. 2000) (see Chap. 8), data from sediment traps need to be corrected for possible under-estimation (or over-estimation) of the 'real flux' (i.e., sampling efficiency) using thorium (^{230}Th, ^{234}Th) or lead (^{210}Pb) isotope based methods (e.g., Scholten et al. 2001; Lampitt et al. 2008; Schmidt et al. 2009; Kuhnt et al. 2013).

Sediment trap samples may be affected by alteration (e.g., dissolution) of particles within the sampling cups. To prevent degradation of the trapped matter, formaldehyde (3–4 %), sodium azide (50 g NaN$_3$ L^{-1}), or mercuric (II)-chlorid (3.3 g HgCl L^{-1}) may be added to poison the sampling vessels (e.g., Fischer and Wefer 1991; Koppelmann et al. 2000; O'Neill et al. 2005, and references therein Buesseler et al. 2007). A buffer (e.g., sodium borate) should be used to keep pH $\geq$ 8.2, to prevent dissolution of calcareous particles including foraminifer tests. Sampling

Table 10.1 Planktic foraminifer analyses from sediment trap deployments from all of the major ocean basins

#	Lat.[N] Long.[E]	Location	Time of deployment	Sampling intervals (days)	Trap depth (m)	Test size (µm)	Data	References
1	21°9′–20°41′	NE Atlantic	4/1990-11/1991	10–21.5	730	n.s.	CaCO$_3$, δ^{18}O	[1]
	20°45′–19°45′	Off Cape	3/1988-3/1989	27	2195		*G. ruber*	
	21°9′–20°41′	Blanc	4/1990-11/1991	10–21.5	~3500		(200–300 µm)	
1	20°45.6′–18°41.9′	Off Cape	7/2005-9/2006	21.5	1277	~420	Mg/Ca, *G. inflata*	[2]
		Blanc					*G. ruber*	
2	28°42.5′–13°9.3′	Canary	1/1997-9/1997	~14	800	<1000	Test flux	[3]
	29°11′–15°27′	Islands	12/1996-9/1997		700			[4]
					900			
					3700			
	29°45.7′–17°57.3′		1/1997-10/1997		4200			
3	33°–20°	Madeira	2/2002-4/2003	5–61	2000	>63	Test flux, species	[5]
		Basin			3000			
			4/2003-4/2004	11–31	3000			
3	33°–22°	NE Atlantic	4/1989-4/1990	14	1000	150–	CaCO$_3$ flux	[6]
					2000	250		
4	47°–20°				1000			
					2000			
					3749			
4	47°50′–19°39′	NE Atlantic	4-9/1992	5–8, 28	~1000	>20	Test and	[7]
			9/1996	14	2000	>100	CaCO$_3$ flux	
			6/1992-5/1993	28	2030	>20		
			5/1992-5/1993	8, 14–28	~3500	>20		
5	54°–21°		6-7/1992	28	2200	>20		

(continued)

Table 10.1 (continued)

#	Lat.[N] Long.[E]	Location	Time of deployment	Sampling intervals (days)	Trap depth (m)	Test size (µm)	Data	References
6	44°33'–2°45'	Bay of Biscay	6/2006-7/2007	5–12	800	>150	Test flux, species	[8]
			6/2006-6/2008	5–12	1700			
7	~45°–6°	Bay of Biscay	7-19/4/2009	0.125	200	>100	Test flux, species	[9]
8	36°1'–4°16'	Alboran Sea	6-1997-5/1998	3–11	1004	>150	Test flux	[10]
	36°14'–4°28'	(Med. Sea)	3/1998-5/1998	3–10	958		Species	[11]
9	43.02° 5.18°	Gulf of Lions	10/1993-1/2006	14, 30	500	>150	Test flux, species	[12]
	42.41° 3.54°	(Med. Sea)						
10	–32°5'–64°15'	Off Bermuda	4/1978-5/1979	30–75	3200	>125	Test flux, species	[13]
			4/1978-5/1984	60 ± 7	3200	>125	$\delta^{18}O$, $\delta^{13}C$	[14]
					3200	>37		[15]
11	10°30'–65°31'	Cariaco Basin	5/2005-9/2008	14	150	>125	$[CO_3^{2-}]$	[16]
					230			
					410			
					800			
					1200			
11	10°30'–65°31'	Cariaco Basin	1/1997-12/1999	14	230	150–355	$\delta^{18}O$, $\delta^{13}C$	[17]
11	10°30'–64°40'	Cariaco Basin	5/2005-3/2008	14	150	>125	$\delta^{18}O$, $\delta^{13}C$	[18]
					230			
12	27°30'–90°18'	Gulf of Mexico	1/2008-5/2009	4–14	700	>150	Test flux, species	[19]
			9/2009-12/2010				Test $CaCO_3$ flux	

(continued)

Table 10.1 (continued)

#	Lat.[N] Long.[E]	Location	Time of deployment	Sampling intervals (days)	Trap depth (m)	Test size (μm)	Data	References
13	13°31′–54°0′	W Atlantic	12/1977-2/1978	98	389	>100	Test flux	[20]
		Station E			988		$CaCO_3$, species	
					3755			
					5068			
14	15°21′–151°28′	Central Pacific	9-10/1978	61	978	>100		
		Station P1			2778			
					4280			
					5582			
15	50°–145° (Station PAPA)	Subpolar N Pacific	9/1982-10/1983	15–16 (11)	1000	>125	Test and mass flux	[21]
					3858			
15	50°–145°	N Pacific Station P	9/1982-10/1985	14	3800	63–1000	Test flux, $CaCO_3$	[22]
16	42.09°–125.77°	off Oregon	9/1987-9/1988	33–87	1000	>125	Test flux	[23]
	42.19°–127.58°			27–85			Species	
	41.54°–132.02°			30-94				
17	39.5°–128°	NE Pacific	3-8/1981	11, 79	1235	>150–1000	Test flux	[24]
			8/1979-6/1980	318	4050	1000		
18	34°14′–120°2′	St.Barbara B.	8/1993-9/1996	14	590	>125	Test flux	[25]
19	33°33′–118°30′	San Pedro	1-7/1988	7	500	180-	Test flux	[26]
		Basin				600	$\delta^{18}O$, $\delta^{13}C$	[27]
20	27°53′–111°40′	Guaymas B.	2/1991-10/1997	14	485	>125	$\delta^{18}O$	[28]

(continued)

Table 10.1 (continued)

#	Lat.[N] Long.[E]	Location	Time of deployment	Sampling intervals (days)	Trap depth (m)	Test size (μm)	Data	References
21	5°21'–81°53'	Panama Basin	12/1979–12/1980	60	890	>125	Test flux, $CaCO_3$	[29]
					2590		fauna	
					3560			
22	~ −30°–73°11'	off Chile	7/1993–6/1994	8–9	2173	n.s.	Test flux, $CaCO_3$	[30]
			11/1991–4/1992	8–9	3497	n.s.	$\delta^{18}O$, $\delta^{13}C$,	[31]
			7/1993–1/1994	8–9	3520	n.s.	Species	[32]
22	~ −30°–73°11'	off Chile	7/1993–6/1998	6–13	2303–2578	>150	Test and $CaCO_3$ flux, species, $\delta^{18}O$	[33]
23	-60°55', -57°6'	Drake	12/1980–1/1981	52	965	n.s.	*N. pachyderma*,	[34]
		Passage			2540		$\delta^{18}O$, $\delta^{13}C$	
			25/1/1981		surface			
	−62°22'–57°59.9'	Bransfield S.	11/1985–5/1986	9, 18	687	<1000		[35]
24	−62°27'–34°46'	Weddell Sea	1/1985–3/1986	11–22	863	n.s.	Total flux, species,	[36]
						<1000	$\delta^{18}O$, $\delta^{13}C$	[35]
25	−64°55'–2°30'	Maud Rise	1/1987–11/1987	16	4456	<1000	Test flux, species,	[35]
	−64°53.3'–2° 33.2'		1/1988–2/1989	16, 32	360		$\delta^{18}O$, $\delta^{13}C$	
	−64°55.5'–2° 35.5'		3/1989–2/1990	18	352			
26	−46° 56°5'	Crozet	12/2004–12/2005	2–28	3195	>63	Test $CaCO_3$ flux,	[37]
	−49°3' 51°30.59'		1/2005–1/2006	2–28	3160		Species	
	−44°29.9 49° 59.9'		12/2004–12/2005	11–28	2000			

Table 10.1 (continued)

#	Lat.[N] Long.[E]	Location	Time of deployment	Sampling intervals (days)	Trap depth (m)	Test size (μm)	Data	References
27	46°45.6' 142°4.2'	S off	9/1997-1/1999	4.25–15.5,	1060	>150	Test flux	[38]
		Tasmania		(24.5)	3850		$\delta^{18}O$, $\delta^{13}C$	[39]
	51° 141°44.3'		9/1997-1/1999		3080			[40]
	53°44.8' 141° 45.5'		9/1997-1/1999		830			[41]
					1580			
28	−44°37' 178°37'	E off	6/1996-5/1997	~ 16	300	>150	Test and $CaCO_3$ flux	[42]
		New Zealand			1000		$\delta^{18}O$, species	[40]
	−42°42' 178°38'		9/1996-5/1997	7–16	300			
					1000			
28	−50°S 171°	Campbell	5/1998-7/1999	9.5, 10	415	>150	Test flux and weight	[43]
	-52°S 174°	Plateau,			442		Species	
		SE off			362			
		New Zealand						
29	46°7.2' 175°1.9'	NW Pacific	6/1993-4/1994	13–31	1412	>125	Test flux	[44]
30	37°24.2' 174° 56.7'		6/1993-4/1994	9–16	1482		Species	[45]
31	30°0.1' 174°59.7'		6/1993-5/1994	13–31	3873			
32	39°59.8' 165°0.1'	NW Pacific	12/1997-12/1998	17.38,	2986	>125	Test flux, species	[46]
33	43°58.1' 155°3.1'		12/1997-12/1998	15.04	2957			
34	50°0.6' 165°1.5'		12/1997-12/1998		3260			
			12/1997-11/2001		3260		Test flux, $\delta^{18}O$, $\delta^{13}C$	[47]

(continued)

Table 10.1 (continued)

#	Lat.[N] Long.[E]	Location	Time of deployment	Sampling intervals (days)	Trap depth (m)	Test size (µm)	Data	References
35	~25° 137°	NW Pacific	12/1997-8/1999	13, 18	917	>125	Test flux, species	[48]
				(2, 6)	4336		$CaCO_3$ flux	[49]
			8/1998-8/1999		1388			
					4758			
36	~39° 147°		11/1997-8/1998		1371			
					4787			
			8/1998-8/1999		1586			
					4787			
36	~36° 147°		8/1998-8/1999	15, 20, 30	1108			
				(16)	5081			
36	~36° 154°		8/1999-8/2000		1191			
					5034			
37	27°23' 126°44'	Ryukyu Island	10/1994-8/1995	17	1000	>125	Test flux, species	[50]
	25°4' 127°35'	Arc and			3000			
37	~28° to 31°N	East China Sea	3/1993-2/1994	1–16	1–1070			[51]
	~123° to 127°N							
38	~22.5° 120°	SW off Taiwan	10-11/2009	3	233	>150	Test flux and weight	[52]
			3-4/2010		250		$\delta^{18}O$, $\delta^{13}C$	
			7-8/2010		816		Species	
39	8°17.5' 108°2'	S off Java	11/2000-11/2002	16–18	2200	>63	Test flux, Mg/Ca,	[53]
	8°16.1' 108°8.5'	S off Java	11/2002-7/2003	(22, 28)	2460		$\delta^{18}O$, $\delta^{13}C$	
40	17°27' 89°37'	Bay of	2/1994-2/1995	14–38	684	>250	Test flux	[54]
	15°32' 89°13'	Bengal			731			

(continued)

Table 10.1 (continued)

#	Lat.[N] Long.[E]	Location	Time of deployment	Sampling intervals (days)	Trap depth (m)	Test size (µm)	Data	References
41	14°25' 64°35'	Arabian Sea	3-4 and 9-10/1996	26	2986	>20	Test $CaCO_3$ flux	[55]
42	10°45.4' 51°56.6'	Somalia Basin	6/1992-2/1993	7–14	1265	>100	*N. pachyderma*	[56]
	10°43.1'53°34.4'				1032	>125	Species, test size	[57]
							Test flux, $\delta^{18}O$	[58]
43	16.8° 40.8°	Mozambique Channel	11/2003-3/2006	21	2250	>150	Test flux and weight	[59]
							$\delta^{18}O$, $\delta^{13}C$, Mg/Ca	[60]
44	27° 3°51'	Walvis Ridge	8/2000-2/2001	8	2700	>150	Test flux, life cycle, species	[61]
								[62]
	27° 3°51'	Walvis Ridge	2/2000-2/2001	8	2700	>150	$\delta^{18}O$, species, test flux, habitat depth	[63]

Some of the traps are discontinuous. Consecutive numbering corresponds to numbers in Fig. 10.2. References (Refs.) in square parentheses are given in the table

References. [1] Fischer et al. (1996), [2] Haarmann et al. (2011), [3] Abrantes et al. (2002), [4] Wilke et al. (2009), [5] Storz et al. (2009), [6] Honjo and Manganini (1993), [7] Schiebel (2002), [8] Kuhnt et al. (2013), [9] Siccha et al. (2012), [10] Bárcena et al. (2004), [11] Hernández-Almeida et al. (2011), [12] Rigual-Hernández et al. (2012), [13] Deuser et al. (1981), [14] Deuser (1986), [15] Deuser and Ross (1989), [16] Marshall et al. (2013), [17] Tedesco et al. (2007), [18] Wejnert et al. (2013), [19] Poore et al. (2013), [20] Thunell and Honjo (1981), [21] Reynolds and Thunell (1985), [22] Thunell and Honjo (1987), [23] Ortiz and Mix (1992), [24] Fischer et al. (1983), [25] Kincaid et al. (2000), [26] Sautter and Thunell (1991), [27] Thunell and Sautter (1992), [28] Wejnert et al. (2010), [29] Thunell and Reynolds (1984), [30] Marchant (1995), [31] Marchant et al. (1998), [32] Hebbeln et al. (2000), [33] Marchant et al. (2004), [34] Wefer et al. (1982), [35] Donner and Wefer (1994), [36] Fischer et al. (1988), [37] Salter et al. (2014), [38] King and Howard (2003), [39] King and Howard (2004), [40] King and Howard (2005), [41] Moy et al. (2009), [42] King and Howard (2001), [43] Northcote and Neil (2005), [44] Eguchi et al. (1999), [45] Eguchi et al. (2003), [46] Kuroyanagi et al. (2002), [47] Kuroyanagi et al. (2011), [48] Mohiuddin et al. (2002), [49] Mohiuddin et al. (2004), [50] Xu et al. (2005), [51] Yamasaki and Oda (2003), [52] Lin (2014), [53] Mohtadi et al. (2009), [54] Stoll et al. (2007), [55] Koppelmann et al. (2000), [56] Ivanova et al. (1999), [57] Peeters et al. (1999), [58] Conan and Brummer (2000), [59] Fallet et al. (2010), [60] Fallet et al. (2011), [61] Lončarić et al. (2005), [62] Lončarić et al. (2006), [63] Lončarić et al. (2007)

vessels should ideally be filled with filtered in situ seawater obtained from deployment depths ahead of deployment, and salt (1 g NaCl L^{-1}) may be added to produce a dense solution, and to prevent leakage and loss of the sampled matter (e.g., O'Neill et al. 2005). After the recovery of the trap, samples should be stored cool, ideally at 4 °C.

First long-term records of the planktic foraminifer test flux of up to almost seven years from off Bermuda have led to an understanding of the seasonal and interannual population dynamics of planktic foraminifers (Deuser et al. 1981; Deuser 1986; Deuser and Ross 1989). Those early projects have stimulated sediment trap studies in all major ocean basins, from the equatorial to polar ocean, and across a wide range of trophic condition from oligotrophic to eutrophic waters (Table 10.1, Fig. 10.2). The Deuser-traps off Bermuda were deployed 35 times at 3200 m water depth between April 1978 and May 1984, i.e. at average sampling intervals of 60 days (Deuser 1986; Deuser and Ross 1989). The Ocean Flux Program (OFP) in the Sargasso Sea off Bermuda has been run for more than 35 years (see also Bermuda Atlantic Time-Series Study, BATS).

The long-term deployment run by R. Thunell's in the Guaymas Basin was operated at fortnightly sampling intervals (Wejnert et al. 2010; see also McConnell and Thunell 2005). The longest time-series of planktic foraminifer test flux over 12 years, from October 1993 to January 2006, were sampled with sediment traps in the Gulf of Lion, in the northwestern Mediterranean Sea (Rigual-Hernández et al. 2012). However, some of the time-series are discontinuous due to malfunction of the sampling gear, and problems while deploying or recovering the traps. In addition, 'swimmers' or any other 'matter' may block the sampling containers, or affect the samples in any other way (e.g., pH changes caused by degradation of organic matter), and may hence impede quantitative analyses.

Sediment traps with very short sampling intervals of 3 h, drifting at 200 m water depth within the same water body, were employed to sample the short-term flux of planktic foraminifers in the southern Bay of Biscay (Siccha et al. 2012). Those samples have revealed small-scale variability of hours and at a local range (patchiness) of planktic foraminifer tests flux, in contrast to large-scale variability
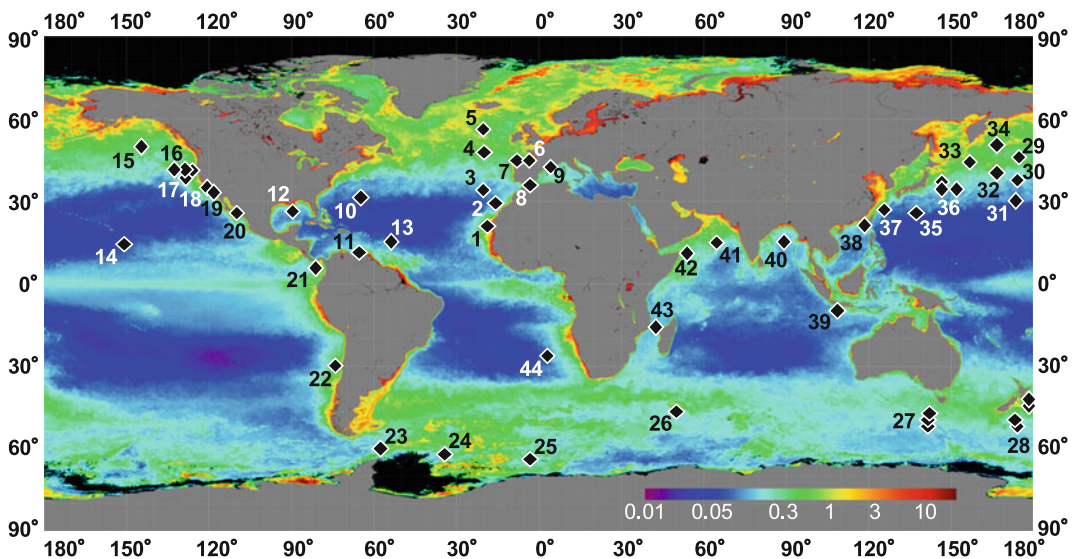
Fig. 10.2 Geographic positions of sediment traps analyzed for planktic foraminifers, and annual global aquatic chlorophyll a concentration (mg m^{-3}, 2013, from Aqua MODIS, http://oceancolor.gsfc.nasa.gov/cgi/l3). Numbering of the trap locations corresponds to Table 10.1

(seasonal to interannual, and regional) investigated by most other approaches (Table 10.1).

Data from sediment trap studies (see also compilations in Schiebel 2002; Žarić et al. 2005, 2006) add information to the systematic understanding of the temporal and regional population dynamics and biogeochemistry of planktic foraminifers (Table 10.1, Fig. 10.2). Although the global coverage of samples includes most of the range of environmental conditions (T, S, productivity) prevailing in the low to high latitude ocean basins (see Fig. 10.2), most remote regions like the central South Pacific have not yet been included in any long-term sampling program due to logistic limitations.

10.2 Processing of Samples

Net-collected samples should be fixed immediately after sampling in a 4 % formaldehyde solution or in alcohol (Ganssen 1981; Hemleben et al. 1989; Schiebel et al. 1995), i.e. addition of one part of concentrated (38 %) formaldehyde to ten parts of seawater sample, or two parts of alcohol to one part of seawater. To prevent dissolution of the shell calcite, wet samples need to be buffered at pH 8.2 using hexamethyltetramine (also called hexamine). Sodium-bicarbonate buffered-formaldehyde solution may be used for sample fixation in case no biogeochemical or morphometric analyses will be carried out, since crusts could precipitate from the bicarbonate-seawater solution, and alter the weight and chemical composition of tests. In case formaldehyde is not available, methyl alcohol or ethyl alcohol could be used for sample fixation. Rose Bengal should not be used to stain the samples (in contrast to processing benthic foraminifers, e.g., Lutze and Altenbach 1991), because the natural color of the planktic foraminifer cytoplasm would be lost, and with it some useful information on the pigmentation of test and cytoplasm. In case samples will not be analyzed for planktic foraminifers (and other calcareous plankton) immediately, buffering needs to be repeated after two month, six month, and from then on once per year, to make up for pH changes caused by degrading organic matter in the sample

solution. Samples should be stored at low temperature (ideally at ~ 4 °C). All steps of preparation and observation should be noted, including changes in storing conditions (e.g., changes in pH, and temperature).

10.2.1 Fixation for Transmission Electron Microscopy (TEM)

Sample fixation for Transmission Electron Microscopy for imaging of the fine structure of the foraminifer cytoplasm is ideally carried out immediately after sampling with a protocol developed by Anderson and Bé (1978). The fixative is minimally disruptive of a wide range of cellular structures, as well as symbiotic algal cells embedded in the cytoplasm. For optimum preservation of the most delicate structures like microtubules, calcium and other interfering substances are excluded from the fixative (Hemleben et al. 1989).

Fixing the cytoplasm of live specimens while stabilizing the molecular structure is achieved with 2 % glutaraldehyde solution in 0.1 M cacodylate buffer (pH 8.2), with 1 % OsO_4 prepared in the same buffer. For optimum preservation of microtubules, the individual should be suspended in a minimum volume of seawater. The fixative should be prepared in a solution at salinity equivalent to the sampled seawater, to exclude as much calcium as possible during fixation.

Following to fixation in OsO_4, the foraminifer shell is removed through decalcification to facilitate subsequent sectioning. In particular, thick shells of mature specimens would disrupt sectioning. To maintain the delicate organic layers during decalcification, specimen are embedded in a 0.8 % agar sol at 40 °C, preferably within a shallow watch glass. The specimen can be isolated within a small (2-mm sized) cube of cold agar, using a line razor blade.

The shell is removed by treating the fixed organisms with 0.1 N HCl or 1 % EDTA (ethylenediamine tetraacetic acid) sufficiently long to remove the shell calcite. The progress of shell dissolution may be monitored with a polarizing light microscope. Alternative to

decalcification, the shell can be dissolved after embedding: The plastic is cut off to expose some surface of shell. The remaining plastic block is immersed in 0.1 N HCl until all calcite is etched away. Subsequently, the block is cleaned with absolute alcohol or acetone to remove any water, re-infiltrated with epoxy, and polymerized.

Dehydration of fixed specimens prior to embedding in a graded series of acetone baths is preferable to alcohol dehydration as precipitation of residual OsO_4 in the specimen is less likely. Dehydrated specimens are embedded in a plastic polymer appropriate in hardness and quality required by the kind of Glas- or Diatome Diamond Knife used to prepare thin sections for TEM analyses.

10.2.2   Analysis of Wet Samples

In the laboratory, wet samples are decanted into a high-rimmed glass dish with a flat bottom, and a diameter sufficient for 'gravity sorting' (e.g., 9-cm Pyrex dish). Heavy particles including foraminifer tests will accumulate in the center of the dish when being carefully rotated. The tests can then be pipetted from the dish under an incident-light microscope of sufficient working distance, using a glass (Pasteur) pipette fitted with a rubber bulb. Tests should be transferred into an evaporation dish made of glass or porcelain. A black microscope table facilitates recognition of the usually whitish tests. Specimens are cleaned from particulate matter using as little water as possible. A minimum of 300 specimens should be enumerated for statistically sufficiently interpretable data (van der Plas and Tobi 1965; Patterson and Fishbein 1989, see Sect. 10.13). Remains of cytoplasm and internal structures of foraminifer tests are particularly well visible in wet samples.

10.2.3   Analysis of Dry Samples

Analysis of dry samples may have advantages over wet analysis, and provides similar results. Before being dried, as much water as possible should be pipetted of the sample. The sample

should then carefully be dried over night at room temperature (~ 20 °C) or in an oven at a maximum temperature of 50 °C. Dry samples are best transferred into 'Franke cells' or 'Plummer cells' with a black background, analyzed, and stored in a dry and clean place for many years. When analyzing very small tests, cardboard cells may be preferred over plastic cells to avoid electrostatic phenomena like any unwanted displacement of tests. A paintbrush may be used for manipulation of the foraminifer tests under the microscope. The finest and most pointed paintbrush should be selected, and which still needs to bear two filaments at the tip to allow for capillary action. Alternatively, a preparation needle can be used. The paintbrush may be used wet (clean tap water will do), the needle with care.

Equal aliquots of large dry samples are produced out with a micro-splitter, also called Otto-micro-splitter. An ideal split contains just above 300 specimens to be classified and counted to produce statistically significant assemblage data (see Sect. 10.13). Assemblage data on entire samples are produced by multiplication of count numbers and split-sizes. Faunal analysis of large samples is alleviated by size-fractionation (sieving) before splitting into aliquots. Sieve sizes of 63, 100, 125, 150, 200, 250, 315, 355, 400 µm, followed by 100-µm increments facilitate comparison of data with other studies. Often applied minimum size classes in assemblage analyses are 100 µm and 150 µm. In addition, size-fractionated samples facilitate balanced analysis of all size classes. In particular, increasingly large and rare tests are sufficiently considered when applying the size-classes given above.

Sieve-size analyses of planktic foraminifer tests usually start at a minimum size of 100 µm for practical reasons. Most plankton nets are equipped with 100-µm gauze, and tests smaller than 100 µm are not quantitatively sampled. Specimens <100 µm in test-size are difficult to classify using an incident light microscope, since those samples include many difficult-to-identify juvenile individuals of large-sized species. Most assemblage studies of planktic foraminifers therefore use the size fractions >100 µm. Tests <100 µm are usually either treated as uniform

size class, or analyzed by means of automated image analyses (see Sect. 10.10).

10.2.4 Wet Oxidation of Organic-Rich Samples

In case samples are too rich in organic matter (e.g., algae or zooplankton) to allow efficient picking of planktic foraminifers for faunal analyses or analyses of test chemistry, oxidation of the organic matter may be advised. For dry oxidation, an oxygen-plasma low-temperature asher (LTA) may be employed. Wet oxidation can also be carried out using standard chemical solutions, i.e. hot (70 °C) 18 % H_2O_2, and 0.024 M NaOH at pH >8, and without any additional technology except of stainless steel sieves, standard laboratory glass ware, and a fume hood (Fallet et al. 2009). In both methods, excess seawater should be removed, and the sample should briefly (to avoid calcite dissolution) be washed with deionized (e.g., MilliQ® water) water to prevent precipitation of salt crystals on the foraminifer tests. While dry oxidation takes about 8 h for LTA alone, the entire process of wet oxidation takes only ∼3 h (Fallet et al. 2009). Both dry and wet oxidation have been shown to not significantly alter weight, stable isotope ratios, and element ratios (e.g., Mg/Ca, Sr/Ca, and Ba/Ca) of the shell calcite of *G. ruber*, *G. sacculifer* (*trilobus* morphotype), *N. dutertrei*, and *G. bulloides* (Fallet et al. 2009).

10.3 Methods in Molecular Genetics

10.3.1 DNA Isolation

The eukaryotic genome is composed of double-stranded desoxyribonucleic acid (DNA) localized in the nucleus and in mitochondria (and chloroplasts in plant cells). The DNA itself consists of the four desoxynucleotides adenine (A), thymine (T), guanosine (G), and cytosine (C). A and T, and G and C, respectively, are bound by hydrogen bonds. For nucleotide sequencing, i.e. for determining the sequence of these nucleotides of a given DNA region, the respective part of interest of the genome is amplified by polymerase chain reaction (PCR) using specific primers. These primers are short desoxynucleotide sequences reconstructed from a known sequence. The isolation of DNA is the initial step, and a necessary prerequisite for nucleotide sequencing. For single-cell foraminifers with a calcareous shell, different methods for DNA extraction have been developed. Merlé et al. (1994) used proteinase K and phenol-chloroform to digest the cells and extract the DNA. Pawlowski et al. (1994) modified the extraction procedure and used a sodium deoxicholate buffer (DOC), a method, which was subsequently applied for most of the foraminifer molecular studies. However, the calcareous shells of planktic foraminifers dissolve in this buffer, preventing any further taxonomic or morphometric classification of the specimens after DNA extraction. Therefore, DOC was later replaced by some workers by a guanidinium thyocyanat buffer (De Vargas et al. 2002), which does not destroy the shells of foraminifers. To date, new methods have been developed making possible the isolation of DNA even after preparation of the cell images (e.g., Seears and Wade 2014).

10.3.2 Selection of Primers and PCR

Before DNA (nucleotide) sequencing, a specific gene region has to be amplified by PCR. For this purpose, flanking primers are designed according to the known conserved regions of the selected gene (e.g., the partial ribosomal SSU (or 18S) RNA gene; see Darling et al. 1997). PCR is carried out with the purified total DNA following standard procedures with subsequent denaturing, annealing and replication steps using a specific DNA polymerase (*Taq* polymerase) isolated from the bacterium *Thermophilus aquaticus,* which is heat-stable and replicates DNA at high temperature. The amplified PCR products are subsequently purified by agarose gel electrophoresis, the respective bands are cut out of the gel, purified, and then either sequenced directly or cloned before sequencing.

10.3.3   Cloning and Nucleotide Sequencing

In order to gain a high-quality sequence read of the PCR amplification products from DNA of a single cell, these are often cloned before sequencing (Grimm et al. 2007; Aurahs et al. 2009b). The PCR products are purified using the QIAquick PCR purification and gel extraction kits (Qiagen) and cloned. For cloning, a PCR product is ligated into a plasmid vector (e.g., pUC18), and transformed into competent *Escherichia coli* cells (*E. coli* DH5α, bacteria strain). Genetic variability within single foraminifer individuals is determined by sequencing several clones. Nucleotide sequencing is carried out in both directions, for example, with an

ABI 377 automatic sequencer (Perkin Elmer) using the standard vector primers M13uni and M13rev. Newly assembled sequences are uploaded to Genbank (http://www.ncbi.nlm.nih.gov/nuccore/), and the accession numbers specify the sequences. The nucleotide sequences obtained are then evaluated by computer analyses. For the different computer programs used in these studies see below.

PCR products can be also sequenced directly. While being considerably faster (from DNA isolation to sequence), the procedure gives rise to replication errors. Therefore, several readings are necessary to obtain the reliable sequence. Direct sequencing can be used if a sequence type is already known from other studies, and large numbers of individuals need to be genotyped.
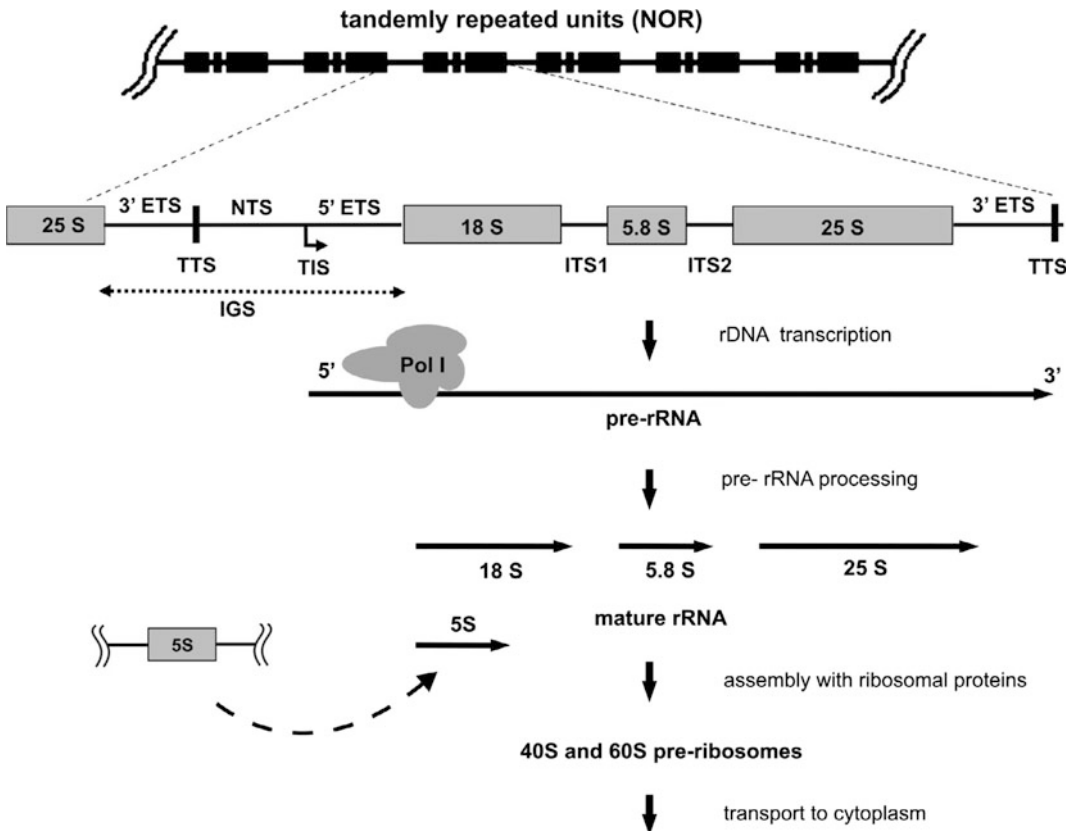
Fig. 10.3 Location and general structural organization of the eukaryotic nuclear encoded ribosomal RNA genes (rDNA) within the nucleolus of the cell nucleus, transcription and processing into the mature rRNA molecules. 18S rDNA corresponds to SSU (small subunit) rDNA, 25//28S rDNA corresponds to the LSU (large subunit) rDNA; ITS, internal transcribed spacer; ETS, external transcribed spacer; TIS, transcription initiation site; TTS, transcription termination site; IGS, intergenic spacer; Pol I, RNA polymerase I; pre-rRNA, rRNA precursor. Modified after Volkov et al. (2007)

10.3.4   Molecular Marker

Ribosomal DNA The nuclear encoded genes (rDNA) are mostly used as molecular marker for the phylogenetic and molecular genetic analyses of Foraminifera. Foraminifer ribosomal RNA genes generally exhibit a similar structure to those from other eukaryotic organisms (Fig. 10.3), although the internal structure of the respective gene regions is highly divergent. The foraminifer SSU (18S) rDNA sequence contains specific variable regions (see below; Hancock and Dover 1988), which can be used for differentiation of species or even different types of "cryptic" species (e.g., Pawlowski and Holzmann 2002; Darling and Wade 2008; Aurahs et al. 2009b; Ujiié and Lipps 2009). Therefore, the SSU rDNA became the standard genetic marker for the characterization of species and different genotypes, and for phylogenetic approaches in planktic Foraminifera. In the meantime, a SSU (18S) rDNA data bank has been established, i.e. PFR^2, Planktonic Foraminifera Ribosomal Reference database (Morard et al. 2015).

The SSU (or 18S rRNA) gene of foraminifers, and particularly of planktic foraminifers, is unique among eukaryotes due to the occurrence of characteristic variable regions 37/e1′, 41/e1′, 45/e1′ and 46/e1′ (Fig. 10.4). The variable region 37/e1′ corresponds to a universal variable region of the prokaryote structure model (De Vargas et al. 1997; Neefs et al. 1990). The other three length-variable regions of the SSU rDNA are also known from the SSU rDNA of other eukaryotes. However, the degree of variability in these gene regions varies greatly between the different groups of foraminifers, and only a few species of planktic foraminifers (e.g. the non-spinose

Globorotaliidae) can be aligned in these regions to benthic foraminifers. For the spinose planktic foraminifers this is only possible within conserved regions of the gene (e.g., De Vargas et al. 1997). Therefore, manual alignments of SSU rDNA of planktic foraminifers were modified based on the SSU rRNA universal secondary structure model (e.g., Van de Peer et al. 1996; Wuyts et al. 2002), in order to include only homologous nucleotide positions in the phylogenetic reconstructions (Darling et al. 2006; De Vargas et al. 1997; Pawlowski et al. 1997; Aurahs et al. 2009b).

In a new approach, the automatical multiple alignment of the sequences gave rather reliable results (Aurahs et al. 2009a). This method has the advantage that the corresponding sequenced gene region, containing both rather conserved and more variable sequences (see Fig. 10.4), can be directly aligned and used for phylogenetic analyses.

Population genetic studies and differentiation of cryptic species can be further defined by using also the even more variable internal transcribed spacers, ITS I and ITS 2, including the conserved 5.8S rDNA, of the rRNA gene (see Fig. 10.3) (Ujiié et al. 2010).

RFLP, Restriction Fragment Length Polymorphism This method allows rapid analysis of a large number of DNA samples from related species or populations. It is faster and cheaper than cloning and sequencing of the respective gene regions. For this purpose, the purified, PCR amplified SSU rDNA products are digested with the respective restriction enzymes at specific short nucleotide sequences, resulting in a number of DNA fragments of different sizes that show genotype specific patterns after agarose gel-electrophoresis (e.g., De Vargas et al. 2001;
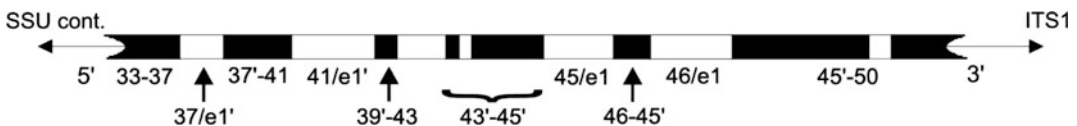

Fig. 10.4 Schematic representation of the 3′ SSU rDNA fragment used for the genetic identification of planktic (and benthic) foraminifers. Black areas represent the relatively conserved regions, white regions correspond to the more variable parts of the fragment. The numbering refers to a hypothetical secondary structure model for the 3′ SSU rDNA according to Wuyts et al. (2002), labeled after the SSU rRNA helices they are encoding for. From Aurahs (2010), modified after Grimm et al. (2007)

Morard et al. 2009). However, it requires previous knowledge of the respective sequences, and minor variations are not detected in the SSU rDNA between closely related genetic types of planktic foraminifers.

Protein-Coding Genes Gene coding for specific proteins have as yet seldom been sequenced and applied to phylogenetic studies of foraminifers. Actin genes, which are rather conserved throughout the eukaryotic kingdom offer a possibility, but have the disadvantage that several paralogs normally exist in the genome, and respective homologs may be compared between different foraminifers (Flakowski 2005).

10.3.5   Next Generation Sequencing (NGS)

With the enormous improvement in nucleotide sequencing methods, rapid increase in knowledge about the genetic constitution and genome evolution of foraminifers is expected in the near future (Pawlowski et al. 2014). Next Generation Sequencing will allow sequencing and comparison of whole genomes. This will facilitate broader information about phylogenetic relationships among different foraminifer species, and verify the occurrence of cryptic species (for a review see Metzker 2010).

10.3.6   Computer Evaluation of the Nucleotide Sequences used for Phylogenetic Studies

ABGD: "Automatic Barcode Gap Discovery" allows calculation of genetic distances within and among genetic types delimitated according to each possible species-level threshold (Puillandre et al. 2012). ABGD is an automatic procedure that sorts sequences into putative species based on a barcode gap, i.e., the gap in genetic distances distribution between intraspecific and interspecific diversity. The barcode gap is observed whenever the divergence among organisms belonging to the same species is smaller than divergence among organisms from different species (André et al. 2014).

GMYC: "General Mixed Yule Coalescent" uses phylogenetic trees to identify transitions from coalescent to speciation branching patterns (Pons et al. 2006). The GMYC approach identifies boundaries between evolutionary units on the basis of shifts in branching rates. Branching within species is the result of coalescent processes, whereas branching between species reflects the timing of speciation events. These methods provide alternative delimitations, and offer the opportunity to analyze sequences that lack former assignation of their genetic type. Finally, these alternative delimitations are confronted in an attempt to connect SSU rDNA sequences to identified genuine species (André et al. 2014).

ML: "Maximum Likelihood", a statistical probability method, is used to estimate the phylogenetic trees for a set of species. The probabilities of DNA base substitutions are modeled by continuous-time Markov chains (Felsenstein 1981, 2004). PhyML trees used for patristic distance calculation, BEAST ultrametric trees and patristic distance matrices (André et al. 2014).

MP: This method in phylogenetics, "Maximum Parsimony" estimates the parameters of a statistical model. It provides estimations for the model's parameters. As an optimal criterion under which the phylogenetic tree has minimized, the total number of character-state changes is to be preferred. The shortest possible tree that explains the data is considered best.

MrBayes: "Bayesian inference (BI)" is a program for Bayesian phylogenetic analysis. The program uses Markov Chain Monte Carlo (MCMC) techniques to sample from the posterior probability distribution (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003).

NJ: "Neighbor Joining" is a bottom-up cluster method for producing unrooted phylogenetic trees based on DNA sequence data. The algorithm requires knowledge of the distance between each pair of taxa (e.g., species or sequences) to form the tree (Saitou and Nei 1987). In contrast, UPGMA (Unweighted Pair

Group Method with Arithmetic mean) produces rooted trees.

PAUP: "Phylogenetic Analysis Using Parsimony" (Swofford 2001).

RAxML: "Randomized Axelerated Maximum Likelihood" is a method used for phylogenetic studies based on large data sets (Stamatakis 2014).

"SplitsTree" is a program for inferring phylogenetic (split) networks (Huson and Bryant 2006).

10.4 Culturing in the Laboratory

Culturing of any biota that serves as proxy in Earth system-science is an indispensable prerequisite for calibration of any proxy (e.g., size, weight, stable isotopes, and element ratios). Planktic foraminifers have been cultured in the laboratory for various analytical purposes since the early 1970s (Bé et al. 1977; Hemleben et al. 1989). Analyses of, for example, radiocarbon (^{14}C) and trace elements (see, e.g., Lea 1999 for a review), boron isotopes (Sanyal et al. 1996), Cd/Ca ratios (Ripperger et al. 2008), and clumped isotopes (Tripati et al. 2010, 2014; Eiler 2011; Wacker et al. 2014) from planktic foraminifer tests requires large sample volumes (several milligrams of $CaCO_3$, i.e. several hundreds to thousands of tests), which could be provided if foraminifer tests could be grown under controlled conditions (e.g., temperature, light) in the laboratory over multiple generations. New designs for culturing of marine micro-biota would allow investigation of planktic foraminifer growth under constant chemical and physical conditions such as, for example, pH, and $[CO_2]$. The rather inexpensive chemostat set-up developed for culturing of benthic foraminifers by Hintz et al. (2004) would potentially allow parallel culturing of large numbers of specimens of any species in time-series experiments (see also Hemleben et al. 1989). A chemostat set-up adopted from the one developed by Hintz et al. (2004) was used for culturing planktic foraminifers (*Globigerina bulloides*, *G. sacculifer*, *G. siphonifera*, *T. quinqueloba*, *N. dutertrei*, *N. incompta*, and *G. inflata*) at JAMSTEC, Yokosuka, Japan (Fig. 10.5).

Culture-protocols are discussed in detail by Hemleben et al. (1989). Culturing of planktic foraminifers has been developed as standard method by H. Spero at the Wrigley Institute for Environmental Science on Santa Catalina Island, California, USA (see, e.g., Spero 1992). The Wrigley Institute for Environmental Science on Santa Catalina Island is situated close to waters where sampling of a variety of abundant live planktic foraminifer species by SCUBA diving is possible. Successful culture experiments are facilitated at laboratories sited close to deep marine waters for sampling of planktic foraminifers, and with infrastructure for culturing experiments. Planktic foraminifers have unfortunately not yet been successfully cultured over an entire generation. Although offspring of *O. universa, G. bulloides, G. truncatulinoides,* and *G. glutinata* have been kept in laboratory culture (e.g., Hemleben et al. 1987; Spero 1992; Spero and Lea 1996; Bijma et al. 1998; K. Kimoto, personal communication, 2007), a second generation has not yet reproduced in culture.

10.4.1 Preparation of Specimens for Culture Experiments

Undamaged specimens should be transferred to the laboratory immediately, i.e. within a couple of hours after sampling. Specimens need to be identified and described using an inverted microscope or incident light microscope. Specimens should ideally be photographed, and transferred to clean culture vessels with the least possible delay. Culture vessels should have a flat bottom to allow for observation with an inverted microscope. Lids of culture vessels may be sealed with Parafilm® to impede gas exchange between culture and atmosphere (Allen et al. 2012). Standard digital cameras are suited for documentation of, for example, chamber formation, changes in cytoplasm color, preservation of spines, gametogenesis, and general behavior under laboratory conditions.
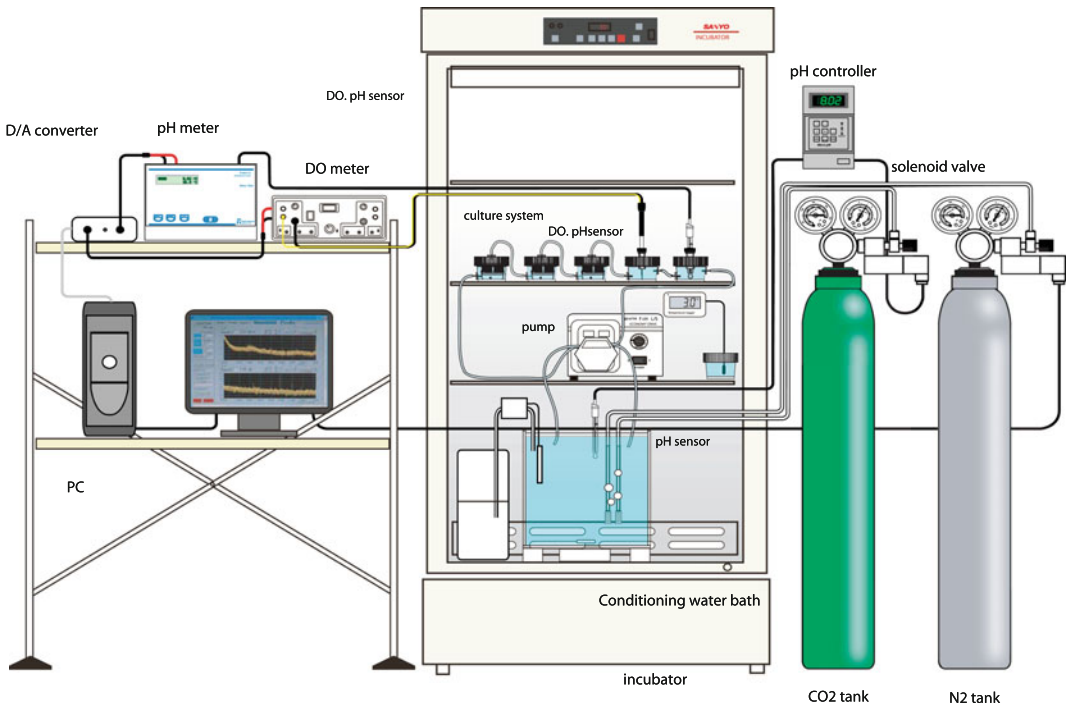
Fig. 10.5 Design of chemostat culture system developed from the design of Hintz et al. (2004). Environmentally controlled (T, S) 20-L seawater reservoir (*light blue*, large) monitored by pH and CO_2 electrodes installed at the top of reservoir. Culture vessels (*light blue*, small) are serially connected. Reservoir and culture vessels are placed in an incubator. Water circulates from the reservoir to culture vessels via Taigon tubes by a peristaltic pump. Taigon tubes protect the culturing water from gas exchange with the outside environment. Culture vessels are closed by screw-lids. The volume of water flow is variable (e.g., 5 mL min^{-1}) to provide equal water quality to all compartments of the culturing system at all times. Circulating water enters culture vessels at the bottom and leaves vessel at the top. To reduce contamination of the system by waste products and particles of all sorts, the inlet of culture vessels is covered with 8-μm gauze. To prevent the incubated foraminifer specimens from escaping culture vessels, the water outlet is closed with a 40-μm mesh. The bottom of vessels is covered with a porous polystyrene cell, which allows constant and balanced water circulation. A 12:12 h dark-light cycle is applied. Light levels within the culture vessel range between 70 and 140 μEinstein m^{-2} s^{-1} (cf. Bemis et al. 1998; Spero and Parker 1985). Culture experiments have been conducted at air-conditioned laboratories at JAMSTEC (Natsushima, Japan). From T. Toyofuku, JAMSTEC, Natsushima, Japan, 2014, with permission

Species should ideally be cultured in their natural ambient seawater, but which needs to be filtered to remove large particles and other plankton organisms (see, e.g., Spero and Williams 1988, 0.45 μm; Allen et al. 2012, 0.8 μm filter). A mix of natural and artificial seawater (1:1) was used to perform a low-DIC experiment (Allen et al. 2012). Filters should be wide enough to not remove fine particles, which could potentially serve as food source for some of the cultured planktic foraminifer species. Water of the culture vessels should preferably be replaced by freshly filtered seawater during days when no food is provided to the foraminifers to not disturb feeding (Spero and Lea 1993).

10.4.2 Feeding in Laboratory Culture

Quality and quantity of food is essential for successful culturing of planktic foraminifer individuals in the laboratory. In addition, chemical and physical parameters need to be carefully chosen and monitored while culturing. Although planktic foraminifers can survive for some time

Table 10.2 Indications of vitality of spinose and non-spinose species, and *H. pelagica* in culture (from Hemleben et al. 1989)

	Healthy	Poorly nourished	Unhealthy
Spinose	Spine length 3× max. test diameter	Reduced spine length	Short spines or no spines
	Network of rhizopodia at or between spines	Same as in healthy individuals	Rhizopodia generally shorter than test diameter
	All chambers filled with cytoplasm	Last formed chamber partially filled or empty	Several chambers only partially filled or empty
	Floating in culture vessel	Same as in healthy	Resting at bottom of culture vessel
H. pelagica	Bubble capsule surrounding test and doubling total diameter	Same as in healthy individuals	Bubble capsule irregular in shape and few bubbles only
	Reddish cytoplasm	Pale reddish to white cytoplasm	Same as in poorly nourished
Non-spinose	Many long rhizopodia may extend at several times test diameter	Same as in healthy individuals	Few short rhizopodia
	All chambers filled with cytoplasm	Final chamber only partially filled with cytoplasm or empty	Several chambers only partially filled or empty
	Attached to and actively moving on bottom of culture vessel	Same as in healthy individuals	Not moving on bottom of culture vessel

without being fed, optimum growth and maturation only occurs if food is appropriately provided (Hemleben et al. 1989). The optimum diet of most species is unfortunately unknown, but both algal and animal prey is consumed by most surface dwelling species (Bé et al. 1977; Anderson et al. 1979; Spindler et al. 1984; Hemleben et al. 1989). Subsurface dwelling species (e.g., *G. truncatulinoides, G. scitula, G. hirsuta*) possibly prefer rather degraded organic matter (Itou and Noriki 2002; Schiebel et al. 2002) at an unknown concentration and quality, which might be the reason for largely unsuccessful culture attempts of any planktic foraminifer species so far.

Most spinose planktic foraminifer species are omnivorous and tend to favor animal prey over algal prey. Adult *G. ruber* and *G. sacculifer* are fed live *Artemia* nauplii, for example, every 48 h (Hemleben et al. 1989; Spero 1992; Allen et al. 2012). The *Artemia* nauplii should not be older than one day. Other foraminifer species may be offered food of different kind or at different frequency (Spindler et al. 1984). Juvenile foraminifer specimens may be fed with small pieces of *Artemia* nauplii (Hemleben et al. 1989). Nauplii food is transferred to the culture dish with a Pasteur pipette and placed near the foraminifer rhizopods, where the food might be accepted within several hours. The feeding process should be monitored, and food might need to be offered several times before being accepted by the foraminifer. Unconsumed food remains need to be removed from the culture dish after feeding (Hemleben et al. 1989).

Non-spinose foraminifer species prefer algal over animal prey, and cultured algae (e.g., *Dunaliella* or *Chlorella*) may be offered to the foraminifers (see Hemleben et al. 1989). In general, appropriate food should be provided at optimal time-intervals to the different species of foraminifers in culture, to keep specimens active and at good health, and enhance the possibility of chamber formation and reproduction (Table 10.2).

10.4.3   Illumination of Symbiont-Bearing Species in Culture

One of the parameters particularly important for culturing of symbiont-bearing planktic foraminifers is an appropriate quality and quantity of light (Jørgensen et al. 1985; Spero and Williams 1988). Illumination may be chosen according to the goal of experiment, and may vary between diurnal 12-h light and 12-h dark cycles, and more or less rapid changes in illumination (e.g., Caron et al. 1982; Jørgensen et al. 1985; Hemleben et al. 1987; Hönisch et al. 2011; Allen et al. 2012). Sufficient light intensity is provided by cool fluorescent light bulbs, and should be monitored with a light meter (e.g., Allen et al. 2012). Compensation light levels where foraminifer respiration exceeds symbiont photosynthesis start at 26–30 µEinstein m^{-2} s^{-1} (Spero and Lea 1993). Maximum symbiont activity occurs at 350–400 µEinstein m^{-2} s^{-1}, and does not significantly increase at higher light levels (e.g., Jørgensen et al. 1985; Spero and Parker 1985; Spero and Lea 1993). Natural illumination at 5–10 m water depth at Barbados during midday in April ranges at 400–500 µEinstein m^{-2} s^{-1} (Caron et al. 1982). In addition to light intensity, the quality of light affects the endosymbiotic activity of planktic foraminifers (Jørgensen et al. 1985), and light sources should be chosen accordingly. Maximum symbiont activity of dinoflagellates in *G. sacculifer* occurs at wavelength of about 450 and 690 nm (Jørgensen et al. 1985).

10.5   Microsensor Analysis

Microsensor analysis of planktic foraminifers was applied to measure photosynthetic rates of symbionts in cultured *G. sacculifer* as early as 1982 by Jørgensen et al. (1985). Oxygen and *p*H were measured with microelectrodes, and manipulated with a micromanipulator at ±5 µm precision. Measurements were carried out under a dissecting microscope, between the spines at the immediate surface of the test of *G. sacculifer*. A similar approach was followed to measure respiration rates of *O. universa* (Fig. 10.6) and *G. sacculifer* (Rink et al. 1998; Lombard et al. 2009a).
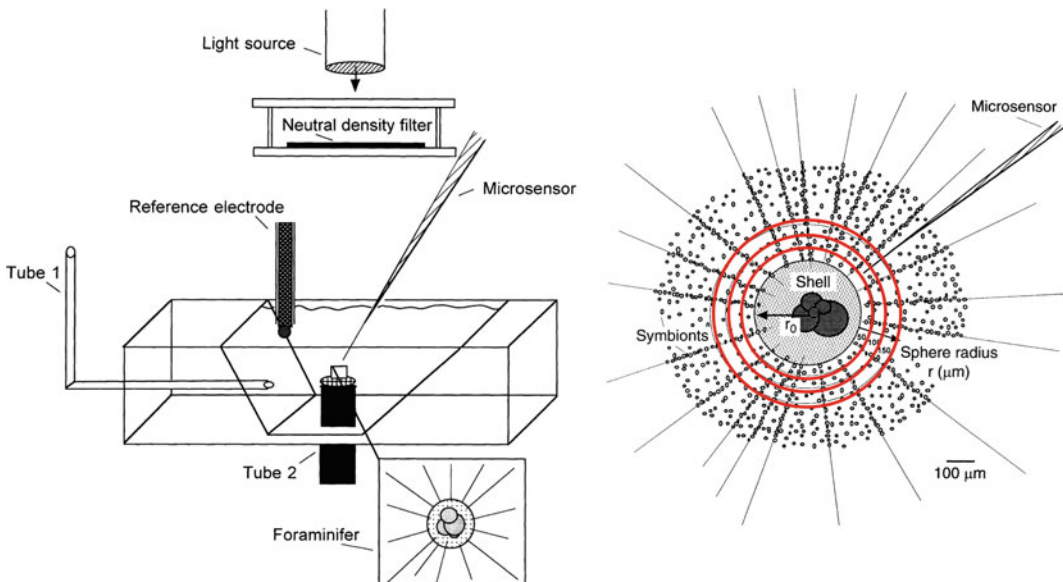
Fig. 10.6 Left panel: Schematic drawing of the measuring chamber (10 mL volume) with a single foraminifer placed on a nylon mesh. Microsensors were positioned with a micromanipulator. The incident light was adjusted by neutral density filters. Right panel: *Red circles* at 50-µm distances indicate microsensor positioning for the photosynthesis measurements (r$_o$ is the radius of a spherical adult *O. universa*). Schematic drawing, after Rink et al. (1998)

10.6 Micro X-Ray Imaging and Computer Tomography (CT)

Micro-CT is a non-destructive method, which provides morphometric information on internal chamber volume, test calcite mass, and dissolution of tests walls (Johnstone et al. 2010, 2014; Görög et al. 2012). Resolution of X-Ray micro-CT ranges between 0.5 and 7 µm depending on the employed scanning system and voltage (Speijer et al. 2008; Johnstone et al. 2010). Experiments in the early 1950s had already shown that microradiography provides sufficient resolution to visualize internal structures of foraminifer tests (cf. Schmidt 1952; Schmidt et al. 2013). Most importantly, X-ray microscopy was employed to visualize the internal test architecture, and the early ontogenetic development of 23 modern planktic foraminifer species (Bé et al. 1969), and several Upper Cretaceous species (Huber 1987). X-Ray diffraction (XRD) was developed to analyze calcite crystallinity of the foraminifer shell as measure of $[CO_3^{2-}]$ (Bassinot et al. 2004).

10.7 Analyses of the Chemical Composition of Tests

10.7.1 Analyses of Stable Isotopes

The ratio of stable carbon and oxygen isotopes, as well as stable isotopes of a suite of other elements of planktic foraminifer shell calcite are major proxies in paleoceanography (e.g., Rohling and Cooke 1999; Fischer and Wefer 1999; Henderson 2002; Katz et al. 2010, and references therein). Pioneering works in the development of mass spectrometry (isotope chemistry) in paleoceanography, and analyses of stable isotopes in foraminifer calcite were started by Epstein et al. (1951, 1953) by developing a paleotemperature equation based on the carbonate of molluscs. Those equations were subsequently refined and applied to foraminifers by Emiliani (1954) when isotope chemistry became an important tool in paleoceanography. Those approaches were then accomplished by N. Shackleton from the 1960s onward (e.g., Shackleton 1968; Shackleton and Opdyke 1973). In addition to paleoceanographic data, stable isotopes add information on the paleo-ecology of planktic foraminifers (e.g., Mulitza et al. 1997; Rohling et al. 2004). For example, from interpretation of the temperature effect on the stable isotope composition of Paleogene planktic foraminifers, Shackleton et al. (1985) could show that depths preferences in the habitat of spinose (globigerinid) and non-spinose (globorotalid) species were opposite from the modern distribution pattern, and Paleogene globorotalids preferred a shallower habitat than globigerinids on average.

Various types of Inductively Coupled Plasma Mass Spectrometers (ICP-MSs) are employed to measure stable isotope ratios from different calcite volumes, and at different reproducibility. Depending on the foraminifer test size and calcite mass, as well as specifications of the employed mass spectrometer, about 3–25 (at least 10 µg $CaCO_3$) specimens are needed for a $\delta^{18}O$ and $\delta^{13}C$ analysis (e.g., Niebler et al. 1999; Rohling et al. 2004). To reduce deviation of results caused by 'vital effects', analysis of stable isotopes should be carried out on mono-specific samples, and from as narrow test size classes of adult specimens (>200 µm) as possible. Standardized analysis of tests of adult individuals reduces the possibility of metabolic effects on the isotope ratio, which can vary significantly between individuals of different ontogenetic stages (Niebler et al. 1999).

Much less volume of calcite is needed in LASER-Ablation Inductively Coupled Plasma–Mass Spectrometry (LA-ICP-MS) and Secondary Ion Mass Spectrometry (SIMS). Between 10 and 100 ng of test calcite are ablated in a helium atmosphere with LASER pulses over some seconds, and measured with an ICP-MS. Test walls ablated by LASER ideally measure some 20–40 µm in diameter, and 0.2–10 µm in depth. Therefore, single chambers of planktic foraminifer tests can be analyzed using LA-ICP-MS (Eggins et al. 2003; Reichart et al. 2003) (Fig. 10.7). Horizontal and vertical (i.e. depth) resolution of (Nano-) SIMS analysis
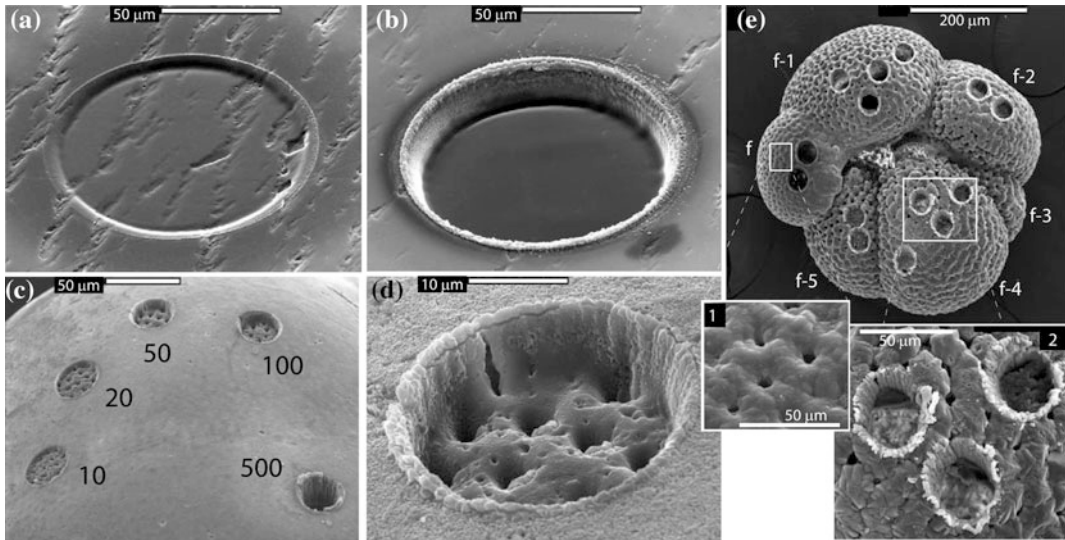
Fig. 10.7 SEM images of LASER ablation pits formed in (**a**, **b**) gem-quality Iceland spar using 10 and 100 LASER pulses, and (**c**) a fossil *P. obliquiloculata* test by 10, 20, 50, 100 and 500 LASER pulses using a LASER fluence of 5 J/cm^2. (**d**) Detail of the 50 pulses pit shown in panel C, which is approximately 7.5 µm deep. (**e**) Test of *N. dutertrei* in which 14 separate composition profiles have been analyzed by LA-ICP-MS and up to four replicates on each chamber. Inset e1 shows detail of the reticulate surface texture present on the final chamber. *Inset* e2 shows detail of 30 µm diameter pits in chamber f-4 and the surrounding blocky calcite textured test surface. Labels f, f-1, f-2, etc. indicate the chamber calcification order, counting back from the final chamber (**f**). Note scale bars. From Eggins et al. (2003)

ranges at 6–10 µm and ∼ 1 µm, respectively (Kunioka et al. 2006; Vetter et al. 2014).

All chemical analyses of foraminifer tests, in particular the high-resolution analyses of small sample volumes, do critically depend on the preparation of samples, i.e. on the cleaning steps to expose the original calcite to be analyzed (e.g., Boyle and Keigwin 1985; Barker et al. 2003; Eggins et al. 2003; Vetter et al. 2013). A flow-through method for cleaning (dissolving) foraminifer tests was developed by Haley and Klinkhammer (2002). The method employs chromatographic equipment, and is assumed to produce reproducible results. In addition, the method provides information on the contaminant phases.

10.7.2   Analyses of Element Ratios

Element ratios of planktic foraminifer test calcite are measured with mass spectrometers such as, for example, Inductively Coupled Plasma-Optical Emission Spectrometers (ICP-OESs; e.g., Friedrich et al. 2012), or Multi Collector ICP-MS (e.g., Fietzke et al. 2004). In general, cleaning methods are similar to those in stable isotope analyses (see above), but might need to account for a much wider range of contaminants depending on the element ratio to be analyzed. High-resolution MC-ICP-MS is employed for analyses of trace elements with very low concentrations (see, e.g., Paris et al. 2014; Ripperger et al. 2008). Ratios of rare elements may also be analyzed applying Thermal Ionisation Mass Spectrometry (TIMS, or isotope dilution TIMS, ID-TIMS) like cadmium-to-calcium ratios (Rickaby et al. 2000). TIMS is widely applied to obtain U-Th ages (e.g., Bard et al. 1993).

Electron microprobe or ion microprobe analysis using an Electron Probe Micro Analyzer (EPMA, or EMPA) allows high-resolution mapping of element ratios of foraminifer test walls (e.g., Duckworth 1977). EPMA is a non-destructive method, widely applied to measure Mg/Ca ratios (Sadekov et al. 2005;

Toyofuku and Kitazato 2005; Kozdon et al. 2011). For EPMA analysis, specimens are embedded in epoxy resin on glass slides, polished to produce a cross section of the test wall, and coated with carbon. The size of each spot-measurement is ∼2 µm. Standard deviation (2σ) is 1.2 % for Mg, and 1.6 % for Ca (Toyofuku and Kitazato 2005). EPMA and LA-ICP-MS data from the same samples are comparable by applying a constant calibration factor (Eggins et al. 2004; Sadekov et al. 2005; Fehrenbacher et al. 2015). A similar resolution of ∼2 µm is achieved with Particle-Induced X-ray Emission (PIXE) in multi-element analysis of planktic foraminifer tests (Gehlen et al. 2004).

Secondary Ion Mass Spectrometry (SIMS, and NanoSIMS) allows measurement of metal-to-calcium (Me/Ca) and stable isotope ratios of planktic foraminifer tests at ∼1–10 µm resolution, and from small sample volumes <2 µg (Bice et al. 2005; Kunioka et al. 2006; Vetter et al. 2014). Tests need to be cleaned, mounted on slides using ethyl cyanoacrylate instant adhesive and low viscosity epoxy resin, and polished to expose the test wall to be analyzed (Bice et al. 2005). An even surface is produced by repeated application of the adhesive and polishing. Samples need to be cleaned between each step by sonication. Standard deviation of replicate Mg/Ca measurements is <1 %. SIMS are in good agreement with ICP-MS data produced from the same samples (Bice et al. 2005; Vetter et al. 2014). Accuracy of $\delta^{18}O$ data from Ion Microprobe analyses is affected by preparation and geometry, as well as instrumental characteristics, and need to be corrected before being compared to ICP-MS data (Kozdon et al. 2009, 2011).

Cleaning protocols: Cleaning of planktic foraminifer tests for analyses of trace metal ratios is essential to generate accurate and reproducible results (e.g., Boyle and Keigwin 1985). To properly clean the tests from the outside and inside, they are gently broken open between two glass slides (e.g., Barker et al. 2003; Sexton et al. 2006). Ultrasonication may be applied with care (for some seconds) to not disintegrate test fragments. Oxide coatings are to be removed in particular for Cd/Ca analyses (e.g., Boyle and Keigwin 1985; Ripperger and Rehkämper 2007). For analyses of Mg/Ca and Sr/Ca ratios, most importantly silicate contamination needs to be removed, as well as clay, Mn-oxides, and Fe-oxides by reductive treatment (Barker et al. 2003). Organic matter is removed by oxidation (Barker et al. 2003). For analyses of live planktic foraminifers from plankton-tow samples the oxidative step using hydrogen peroxide may be repeatedly applied to entirely remove cytoplasm from within the tests. In turn, the reductive and oxidative steps may be omitted because they may remove significant portions of calcite from shell surfaces (Vetter et al. 2013). The reducing reagent alone may causes partial dissolution of carbonate resulting in up to 15 % reduced Mg/Ca values on average compared to studies without reductive step (see in-depth discussions by Barker et al. 2003; Sexton et al. 2006; Bian and Martin 2010). To remove any re-adsorbed contaminants, a final weak acid 'polish' may be performed (e.g., Friedrich et al. 2012).

Calibration for temperature calculation from Mg/Ca ratio: Species-specific calibrations are applied to calculate ambient seawater temperature from the Mg/Ca ratio planktic foraminifers. Calibrations are available for *G. bulloides* and G. ruber from Elderfield and Ganssen (2000) and Anand et al. (2003), respectively. For other species, the multi-species calibration of Anand et al. (2003) may be applied (Friedrich et al. 2012). Those calibrations indicate a temperature sensitivity for Mg/Ca of ∼10 % for a 1 °C change in temperature for almost all planktic foraminifer species (e.g., Anand et al. 2003; Elderfield and Ganssen 2000; Lea et al. 1999).

10.7.3 Radiocarbon Analyses

Radiocarbon (^{14}C) is measured from planktic foraminifer tests for absolute dating of late Quaternary sediments (e.g., Bard 1988; Voelker et al. 1998; Barker et al. 2007). About 800–1000 tests of medium sized ($\sim$250 μm in test diameter) planktic foraminifers equal 10 mg of calcite needed for one ^{14}C Accelerator Mass Spectrometry (AMS) measurement (e.g., Voelker et al. 2000). Although surface dwelling planktic foraminifers produce their test calcite in relative vicinity to the atmospheric ^{14}C pool, large deviation of their ^{14}C AMS signal from calendar ages have been detected (e.g., Reimer et al. 2013). Those deviations result from reservoir effects, i.e. the age of ambient water body in which test calcite is precipitated. Consequently, radiocarbon should preferably be analyzed from mono-specific samples, since different planktic foraminifer species may calcify their tests at different water depths, different seasons, and different ecologic conditions, i.e. in waters of different age. Depending on ocean basin and region, the most abundant species may be selected for ^{14}C AMS analysis, still taking its ecology into consideration. For example, *G. bulloides* are most frequent in high-productive waters like upwelling regions, i.e. waters with relatively old ^{14}C AMS ages, and high reservoir ages. In contrast, *G. ruber* is more productive in waters marginal to upwelling cells and more stratified surface waters (e.g., Schiebel et al. 2004), and would hence represent waters of lower reservoir age. In case a sufficient amount of mono-specific tests is not available from a sample, tests from species with similar ecologies could be combined for ^{14}C AMS dating. In addition to species-specific, as well as regional and seasonal differences, reservoir ages change over time (e.g., Bard 1988; Reimer et al. 2013). To account for all of the different effects, which affect the ^{14}C AMS age of planktic foraminifer calcite (Barker et al. 2007; Mekik 2014, and references therein), and which cause deviation from calendar age, raw radiocarbon data need to be calibrated (Reimer et al. 2013, and references therein).

10.8 Biomass Analysis

A non-destructive method for biomass analysis of individual foraminifers was developed and calibrated by Movellan et al. (2012). The method employs nano-spectrophotometry and a standard bicinchoninic method for protein quantification (Smith et al. 1985), assuming that foraminifer protein-biomass equals carbon-biomass (Zubkov et al. 1999; Movellan 2013). Following protein measurement, tests are dried and stored for fuurther analyses.

Foraminifer individuals are isolated immediately after sampling. Each individual is transferred into a bath of micro-filtered seawater, and gently cleaned with a brush to remove particles. Specimens are then immersed in deionized water for less than a second to remove remaining seawater. Each foraminifer is individually stored in an Eppendorf cup and immediately analyzed for biomass, or stored frozen at −80 °C to prevent degradation of organic matter, and facilitate later protein-biomass quantification.

For biomass analysis, 20 μl of micro-filtered tap water is added to each Eppendorf cup including fresh or unfrozen foraminifers for 30 min. Immersion of foraminifers in micro-filtered tap water causes an osmotic shock, and quantitatively exposes the foraminifer cytoplasm to the working reagent (400 μL), which is then added to each Eppendorf cup (Movellan et al. 2012). Efficiency and yield of the osmotic shock method for cytoplasm exposure was tested on specimens of *Globorotalia hirsuta*, *Globorotalia scitula*, and *Globigerinella siphonifera*. The three species were chosen for their differences in test architectures, i.e. globular chambers with wide apertures (*G. siphonifera*), compressed chambers with intermediate-sized apertures (*G. hirsuta*), and compressed chambers with small apertures (*G. scitula*).

Protein-biomass analyses with the bicinchoninic acid (BCA) method employ a mix of copper solution (4 % (w/v) $CuSO_4$ 5H_2O solution; Sigma-Aldrich) and BCA (Sigma) solution (Smith et al. 1985; Zubkov and Sleigh 1999; Mojtahid et al. 2011). In contact with proteins the Cu^{2+} ions of the copper solution are reduced to Cu^+. The Cu^+

ions react with the BCA, and a purple color is produced. The intensity of the color increases proportionally with the protein concentration. Protein standard solution consists of bovine serum albumin (BSA) of known concentration. Each sample and standard solution is measured in triplicate (Movellan et al. 2012). Foraminifer samples and protein standard solutions are prepared simultaneously, to make sure that the incubation time and temperature are identical. The reaction and resulting coloration of the sample solution depends on incubation time and temperature. An optimum color spectrum is obtained at an incubation time of 24 h at room temperature (20 ± 2 °C).

After incubation, each sample is centrifuged for 3 s at 5000 rpm, and the absorbance of the 562-nm wavelength is measured with a nano-spectrophotometer on 2 µL of sample or standard solution (NanoDrop 2000®, Thermo Scientific). The absorbance of the working reagent is affected both by color and brightness resulting from the concentration of proteins. Each absorbance value is measured three times, and standard curves are constructed using polynomial regression.

10.9 Determination of Test Calcite Mass

Calcite mass of planktic foraminifer tests is a measure both of production and dissolution of shell, and hence provides information on environmental conditions of ambient seawater of live individuals, and settling tests (e.g., Barker and Elderfield 2002; de Moel et al. 2009; Moy et al. 2009). Among the parameters affecting production and remineralisation of test calcite are, in final consequence, carbonate chemistry ($[CO_3^{2-}]$, and other carbon species) and pH, which are affected by light and symbiont activity (i.e. $[CO_2]$) in the symbiont-bearing foraminifer species. Therefore, different methods were developed to determine planktic foraminifer calcite mass.

The most obvious method appears to be simple weighing of clean, empty, and well-preserved (i.e. unbroken) tests of similar ontogenetic stage. To produce comparable results, size-normalized test weights are determined (Lohmann 1995; Broecker and Clark 2001a, b; Beer et al. 2010a, and references therein). Batches of tests from narrow size intervals (e.g., 200–250 µm) may be produced by sieving. To compensate for any variability in size and mass of tests from the same sieve-size interval, a sufficient number of tests (e.g., 10–50 tests) may be combined for weighing (Broecker and Clark 2001b). Alternatively, tests may be analyzed for their discrete size and weight (Broecker and Clark 2001a, b). Both methods are inexpensive and fast, and produce interpretable results.

A microbalance (e.g., Mettler Toledo XP2U, readability of 0.1 µg) may be employed to weigh individual foraminifer tests, or batches of tests (Moy et al. 2009; Movellan et al. 2012). Weighing should be carried out after a minimum of 12 h of acclimatisation in an air-conditioned weighing-room at constant temperature and humidity. Repeated weighing (three times) of individual foraminifer tests (>100 µm) is advised to enhance precision of data (Schiebel and Movellan 2012).

Unfortunately, fossil tests are often filled with sediment, and impossible to clean without causing damage to the original shell. Therefore, methods independent of test size and weight were developed to determine shell calcite mass. Crystallinity of test calcite as measure of dissolution is analyzed using X-ray diffraction (Bassinot et al. 2004). The method provides quantitative results for past $[CO_3^{2-}]_3$, given that conditions of production and sedimentation are analogous to modern conditions (Bassinot et al. 2004). Measurement of shell-thickness of equivalent cross-sections (i.e. of the same species, and same chamber) with a Scanning Electron Microscope (SEM, see below) provides information on calcite mass (de Moel et al. 2009), but would possibly not be suited for analyses of large sample volumes, since rather time-consuming and costly.

Shell calcite mass determination: A variety of different methods have been developed for the determination of the planktic foraminifer test calcite mass as proxy of shell production and dissolution. (1) Weighing seems to be the most obvious method, but it is limited by the precision of weighing balances within the range of 0.1 µg at the best, and the weight of small tests (<100 µm) below 0.6–1.2 µg even for well preserved modern specimens (Schiebel and Hemleben 2000; Barker and Elderfield 2002; Schiebel et al. 2007). Weighing, hence, would not be suitable to detect differences between individual small tests, which are calcified or dissolved to a different degree. In addition, any kind of contamination within, or on the surface of, the test, and any sediment infill, would not be detected by weighing. The same would possibly be true for any titration method. (2) Analyses of the crystallinity of the planktic foraminifer test calcite, inferred from X-ray diffraction, requires crushing of a large number of tests; i.e., for example about 80 *G. ruber* of the 250–315 µm size fraction (Bassinot et al. 2004). Analysis of crystallinity by particle-induced X-ray emission (PIXE) requires only single tests, but is nonetheless a destructive and laborious method (Gehlen et al. 2004). Therefore, application of the method is limited by the availability of tests, as well as manpower. (3) X-ray computed tomography (CT) provides images from the outside and inside of the tests at a resolution of 7 µm. Taking only ∼50 min per specimen for CT scanning, the method is still not suited for analyses of entire assemblages (Johnstone et al. 2010). (4) SEM analyses are suited to visualize encrustation and dissolution of the primary shell calcite at high detail, but this method requires expensive technology, and possibly cannot be quantitatively applied to assemblages, because it is too costly. (5) A combination of some of the above given methods may be suited to resolve the test-calcite-mass problem to a satisfactory degree.

10.10   Automated Microscopy

Microfossils have played a key role in palaeoceanographic reconstructions, largely as proxies of changing water mass properties traceable by their faunal and stable isotopic compositions and their trace-element chemistry. Although major effects on the population structures and evolutionary developments of associated assemblages are expected, little work has been done so far, largely because of the time-consuming morphometric and taxonomic data collection. This problem has been overcome by automated acquisition and processing of data (Schmidt et al. 2003; Bollmann et al. 2004, and references therein; Schmidt et al. 2004a, b, c; Beer et al. 2010a, b).

Automated particle analysis in palaeoceanography and micropalaeontology is carried out with a fully automated incident light microscope system (Bollmann et al. 2004). Images are acquired and particles are analyzed with analySIS FIVE (SIS/Olympus©) software supported by a custom made software add-in. Samples are prepared on up to six glass trays, and are automatically moved under a Leica© Z16APO monocular microscope with a plan-apochromatic objective using a motorized xy-stage and Lstep-PCI controller manufactured by Märzhäuser© (Germany). Manual positioning of the xy-stage with a joystick for analyses of particular objects is also facilitated via analySIS. Images are captured with a 12-megapixel CC12 colour camera (SIS©). Constant illumination of samples is provided by a Leica© CLS100X light source and a Leica© ring-light (Clayton et al. 2009). Resolution of the system ranges from 1.44 × 1.44 µm to 24.5 × 24.5 µm per pixel. Depending on average particle size, between ∼2000 and ∼10,000 particles per sample tray can be analyzed
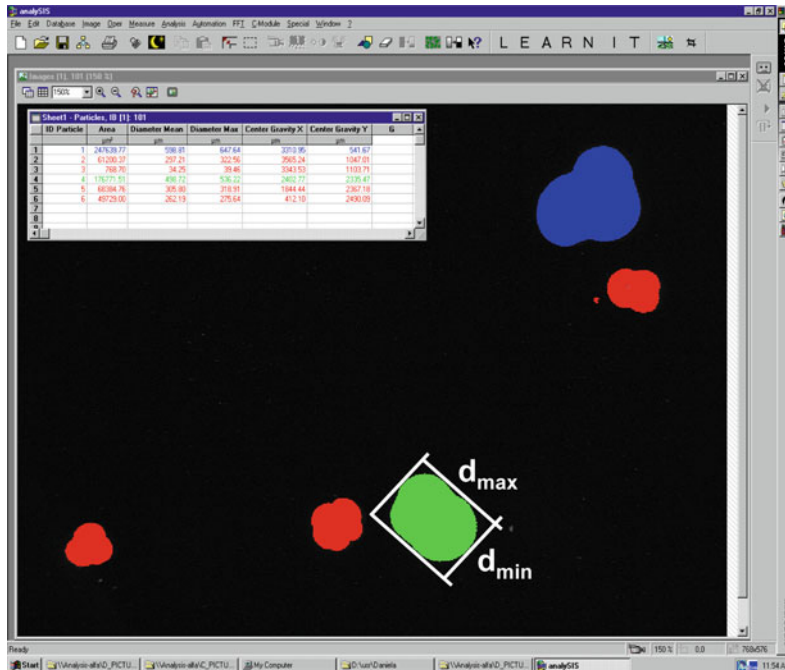
Fig. 10.8 Screen-shot of automated image analysis system (analySIS FIVE, SIS/Olympus©). Planktic foraminifer tests are sorted for size (color scheme) in the example shown here. Minimum test diameter (d_{min}) rectangular to maximum test diameter (d_{max}) acquired automatically. An additional 111 morphometric measures and color parameters can be automatically acquired by the system from image series obtained from up to six strew mounted samples at high efficiency

(Schmidt 2002). Acquisition of images and morphometric analyses of the images takes between 15 min and 1.5 h per sample, respectively. Each particle can be analyzed for up to 65 morphometric parameters, 29 color and gray-scale parameters, and additional 20 parameters to be user-defined (analysis FIVE, SIS/Olympus©) (Fig. 10.8). The data are automatically saved, for example, as an Excel spreadsheet.

Minimum test diameter: The minimum test diameter is applied as a measure of test size, which is easy to acquire and robust. The minimum test diameter is therefore acquired and applied in morphometric analyses of test assemblages. Minimum test diameter is the longest (!) distance measured rectangular to the line of maximum diameter of the test, whereas maximum diameter of the test is the longest distance of the two-dimensional silhouette-area of the entire test (Fig. 10.8). Minimum diameter is a more robust size-measure of the test than maximum diameter, and is therefore used in most morphometric analyses discussing test-size. Minimum diameter does well display test size, being highly correlated to (two-dimensional) silhouette area, i.e., the way tests are viewed from above through a binocular microscope, and which is a good representative of test volume (Beer et al. 2010a). In addition, minimum test diameter

is comparable to sieve-size, since particles including foraminifer tests pass through the mesh of a sieve with their smallest diameter.

10.11 Electron Microscopy

Scanning Electron Microscopes (SEMs) and Transmission Electron Microscopes (TEMs) of various makes are used to analyze fine structures of test and cytoplasm, respectively. Classical SEM and Environmental SEM (ESEM) are employed for high-resolution imaging of hard surfaces, i.e. tests. Tests are analyzed in near vacuum conditions, and hence need to be dry. Classical SEM allows high-quality imaging at high resolution of up to about 1 nm. Objects need to be coated with graphite, gold, platinum, or other conductive materials, though, and may not further be used for chemical analyses. In turn, coating of objects in ESEM is not necessary, and objects stay unchanged during scanning. ESEM is a non-destructive imaging method, which may be employed if objects are to be further used, for example, for stable isotope or element analyses. ESEM can even be employed on wet objects, because vacuum conditions are not applied. In turn, resolution of high-quality images in ESEM is much lower than in classical SEM, and limited to objects >1 μm.

Transmission Electron Microscopy (TEM) is applied for visualization of cytoplasmic fine structures, at a resolution of several nanometers. The valid visualization of delicate and labile cytoplasmic components requires fixation of the live matter in as natural a state as is possible. Following fixation of the cytoplasm (see above), the shell is removed for subsequent sectioning. Dehydrated specimens are then embedded in a plastic polymer appropriate in hardness and quality required by the kind of Diatome Diamond Knife used for sectioning, and the degree of stability needed during examination with the TEM.

10.12 Modeling

Numerical Modeling of planktic foraminifers follows different avenues to better understand physiology and population dynamics, and finally the biology and ecological needs of modern species and assemblages, and the effect of planktic foraminifers on the marine carbon turnover (biogeochemical modeling). Another approach including sensitivity analyses (Žarić et al. 2005), and modeling ('prediction') of the species richness and diversity, relative abundance of species, and test flux, uses empirical input data from sediment traps and surface sediments (Žarić et al. 2006). Modeling of the global distribution and seasonal bias of surface dwelling species in fossil assemblages using a dynamic ecosystems approach is targeted at a better understanding of planktic foraminifers in paleoceanographic records (Fraile et al. 2008, 2009a, b).

Ecophysiological modeling has been empirically based, utilizing input data from laboratory observations and natural distributions of live individuals, and aims at a more complete qualitative and quantitative use of planktic foraminifer as proxy in paleoecology (Lombard et al. 2009b, 2011; Roy et al. 2015).

Modeling in planktic foraminifer research had started much earlier, though. A 'computer method' to calculate planktic foraminifer test architecture and shell growth from simple spheres was designed in the late 1980s (Ott et al. 1992; Signes et al. 1993; Łabaj et al. 2003; Tyszka and Topa 2005). The model includes assumptions on allometric shell growth, protoplasmic growth, and ontogeny of planktic foraminifers, and was designed with a biogeochemical perspective, i.e. to explain the carbon budget of planktic foraminifer shell calcite and biomass (Signes et al. 1993). A following empirical model of planktic foraminifer carbonate flux in the central Red Sea includes biological and ecological information, such as reproduction rate and length of the reproductive period at the species level (*G. sacculifer*). Final goal of the approach was to enumerate calcite flux pulses, and to quantify annual calcite budgets (Bijma et al. 1994).

10.13   **Census Data for Assemblage Analysis**

Assemblages of live individuals or empty tests are analyzed for population dynamics by counting a certain number of individuals. Those analyses are preferably conveyed at the species level, or at a higher systematic level (i.e. morpho-types) if possible. In case of standard counts carried out with an incident light microscope 80× to 120× magnification, planktic foraminifer assemblages are analyzed for morpho-types or morpho-species. The number of individuals to be counted depends on the number of morpho-species in a sample, their relative abundance, and the level of statistical significance and confidence to be achieved. It is generally suggested to count at least 300 specimens per (whole) sample, i.e. all test-size fractions of an entire sample or a representative split of a sample (Patterson and Fishbein 1989).

For example, in case 300 specimens are counted from a sample, and the relative abundance of any species is found to be 15 %, the corresponding value of 2σ is 4 %. The relative abundance of the species hence ranges at 15 ± 4 %, i.e. between 11 and 19 %, at a 95 % confidence (van der Plas and Tobi 1965; Patterson and Fishbein 1989). The relative significance of data increases with increasing relative abundance of a species (>15 %), and decreases with decreasing relative abundance (<15 %). Statistically interpretable data are limited to about 4 % when counting 300 specimens, and to about 2 % when counting 500 specimens (Fig. 10.9). For reasonable interpretation of the distribution of rare species, large numbers of specimens need to be classified and counted, a task, which is rather time consuming. Automated methods in microscopy and image analysis have been developed to speed up and facilitate otherwise time consuming analyses (see chapter on Automated Microscopy above).

Species diversity is one of the basic measures of assemblages, which can be deduced from count data. The simplest measure of diversity is 'species richness', i.e. the number of species in a sample. 'Species richness' does neither account
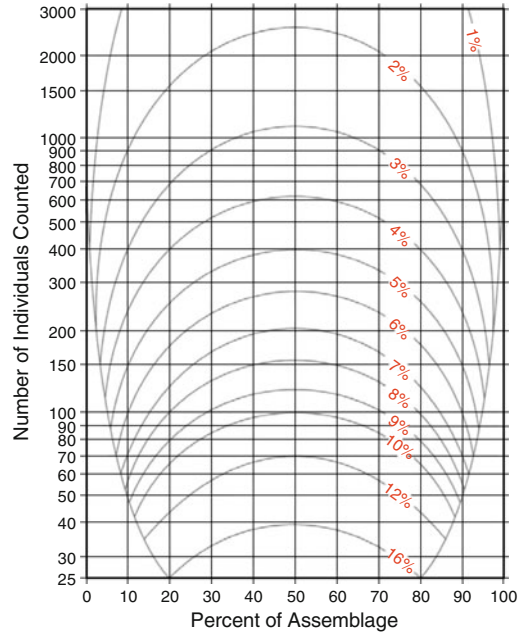
Fig. 10.9 Reliability of test counting results. Curves with percentages give 2σ values. At 300 tests counted, lowest interpretable numbers range at 4 %, i.e. 4 ± 2 % at 50 % rel. σ). Fields in the lower right and left corner are not valid. Redrawn after van der Plas and Tobi (1965)

for the size of sample, i.e. number of specimens counted in total, nor for the relative frequency of species within the sample. A more complete description of species diversity is provided by indices such as the Fisher α index, and the Shannon-Wiener index.

The Fisher α index is used to assess species diversity in a sample, and to estimate species diversity of large samples from numbers obtained from smaller sub-samples (Fisher et al. 1943; Murray 2006). The same is done in ecology by the 'rarefaction' method to assess species richness ('rarefaction curves'). Rarefaction curves are produced by continuously plotting the numbers of specimens of each classified species while counting.

The Shannon-Wiener index (H') is easy to calculate, and in combination with the 'evenness' (E) provides a rather complete description of diversity (Shannon 1948; Shannon and Weaver 1963; Hayek and Buzas 1997).

$$H' = -\sum_{i=1}^{n} p_i \ln p_i \quad (i = 1 \text{ to } n) \qquad (10.1)$$

with p_i being the proportion (numbers ≤ 1, i.e. per cent divided by 100) of the ith species in a sample, and ln being the natural logarithm. H' hence combines information on the number of species present in a sample, and the relative abundance of species. Similar H' may be produced by different combinations of species distributions. For a complete and unequivocal description of species diversity in a sample, 'evenness' ($0 \geq E_H \leq 1$) or 'equitability' provides a measure of the balance of the distribution of species in a given sample, with S being the total number of species present in a sample.

$$E_H = H'/H_{max} = H'/\ln S \qquad (10.2)$$

10.13.1 Statistical Analysis of Assemblage Data

Simple linear regression of least squares is the basic statistical method applied for the comparison of data. Resulting correlation coefficient, standard error, standard deviation, and probability (p-values) are used for a statistical description of the distribution of data. Student's t-test and F-test are applied for the comparison of two populations of data. In case more than two populations data are to be compared, an Analysis of Variance (ANOVA) may be carried out.

Multivariate analyses may be employed to group information from data, and to visualize information of large and complex data sets. Cluster analyses produce dendrograms, in which data are grouped in clusters. Differences between clusters (i.e. groups) are expressed as distances. Multidimensional correlation of data is called factor analysis. Factor analysis produces groups of data called factors. Factors and clusters may be produced by different methods, and by the use of different algorithms. Software packages allow easy application of multivariate methods, and production of multivariate data. In turn, interpretation of the resulting data might be more difficult than the production of results, and it is strongly advised to seek the help of an expert for reasonable interpretation of data.

10.13.2 Analyses of Test Size Data

Ontogenetic development of planktic foraminifer tests occurs at intervals by adding new chambers to the test. Test size of individuals, and size distribution of assemblages may hence be analyzed either from sieve-size classes or discrete size data (Peeters et al. 1999; Schiebel and Hemleben 2000; Schmidt 2002; Beer et al. 2010b). To account for smaller test-size increments when adding smaller chambers at earlier ontogenetic stages, and larger increments later in ontogeny, sieve-size intervals should increase with foraminifer test size (see above). Discrete size measurement such as, for example, from image analyses, provides more detailed data than sieve-size analyses. However, sieve-size effects may be averaged out when large numbers of specimens are analyzed. In addition, any methodological affects caused by sieving, and physical damage of tests, are largely avoided in image analyses.

Size-distributions of planktic foraminifer test assemblages are inherently incomplete to some degree for test-sizes close to the sampling mesh-size (e.g., ≥ 100 μm). Small specimens near the sampling mesh-size may be missed, and very small specimens just below the sampling size may be included. The latter are easily identified during later analytical steps, and may be excluded from further analyses. Missing of the former may be detected by cohort analysis: The number of individuals should increase with decreasing size (Fig. 10.10), or decrease to a reproducible degree (Peeters et al. 1999). If this is not the case, and the smallest sampled size-class contains fewer individuals than the second smallest size class, a methodological (sampling) error may be the reason (Schmidt 2002). An introduction to the theoretical background of natural, i.e. biological and ecological effects on body size is given by Schmidt et al. (2006).
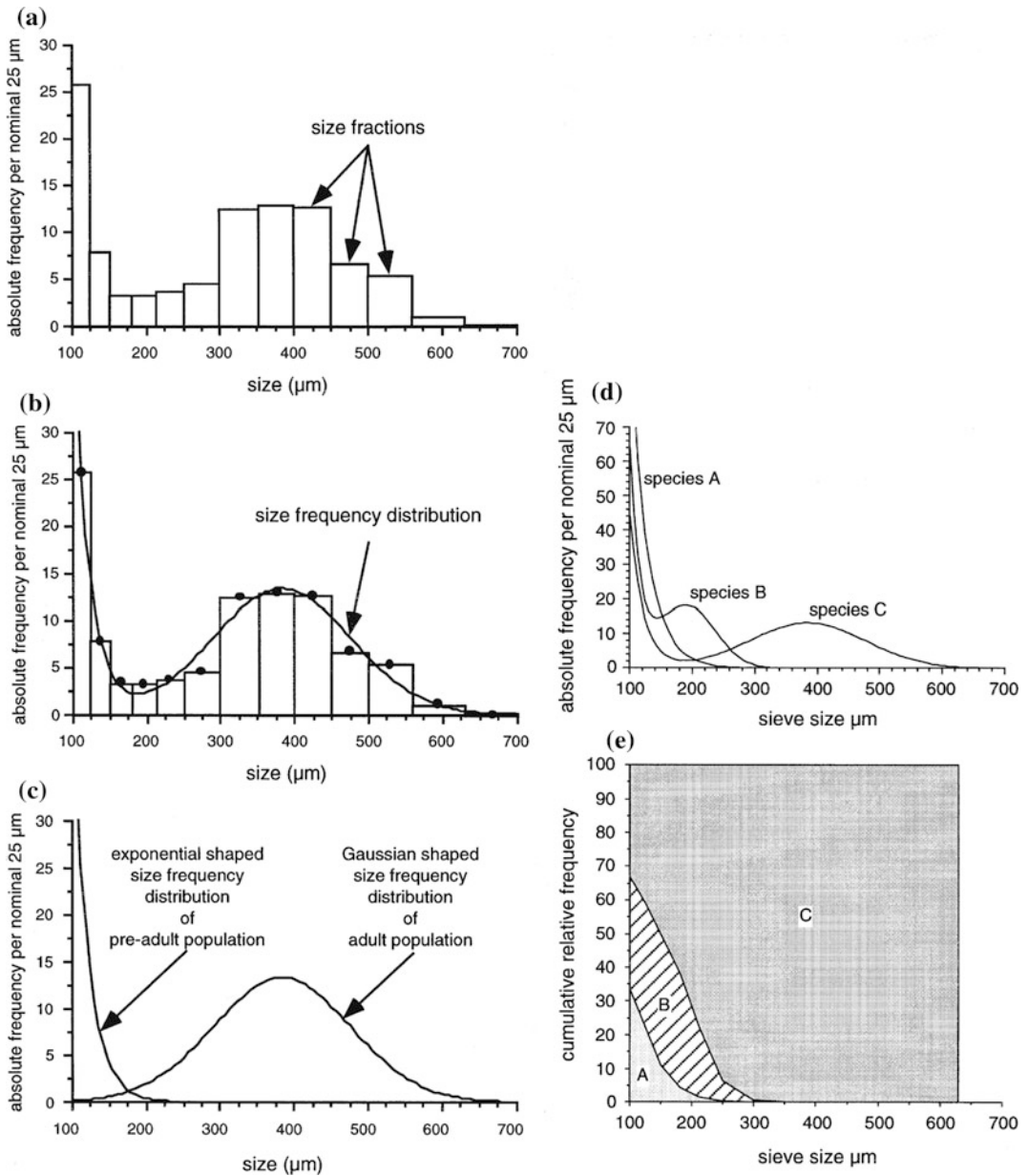
Fig. 10.10 Interpretation of size frequency distributions of planktic foraminifer species and assemblages from plankton net samples, sediment traps, and surface sediments. **a** Frequency normalization per size fraction may be applied if widths of size fractions are not equidistant. **b** Curve fitting to obtain a size frequency distribution in large assemblages. The size of any foraminifer species within a size fraction may finally be represented by a single value, i.e. the mean of all size fractions. **c** Exponential and normal distribution may explain 'hidden' cohorts in count data. The sum of both cohorts yields the size frequency distribution of the whole assemblage. **d** Cohorts of three species (A, B, C) with different size-frequency distributions, caused by (**e**) differences in relative abundance and test size within a hypothetical sample. After Peeters et al. (1999)

When analyzing test-size data at the species level, it should be accounted for mortality rates and reproduction rates of pre-adult and adult cohorts, respectively (Schiebel et al. 1995; Peeters et al. 1999). Assemblage size-analyses may include size-effects caused by both cohorts of the same species, and size differences between species (Fig. 10.10). Bimodel or polymodal size-distribution of the same species within a sample may also indicate mixing of populations, and expatriation/immigration of individuals by currents. The same is true for size-sorted assemblages, which may be cut off at either side, and hence lack either small (juvenile) or large (adult) individuals.

10.13.3 Transfer Functions

Transfer functions are a suite of statistical methods used in paleoceanography to reconstruct past environmental conditions from the distribution of microfossils (e.g., Imbrie and Kipp 1971; Hutson 1977; Sachs et al. 1977; Vincent and Berger 1981; Fischer and Wefer 1999; Guiot and de Vernal 2007, and references therein). Planktic foraminifers are employed in transfer calculations due to their wide distribution, and relatively well-known paleo-biogeography in relation to modern distribution patterns and environmental parameters. Transfer functions have classically been used to reconstruct Sea Surface Temperature (SST). Today, transfer calculations are also employed to reconstruct any other (paleo-) environmental parameter, which is sufficiently resolved in the paleo-record (i.e. down-core) and modern assemblages (i.e. regional coverage of surface sediment samples), provided sufficient sensitivity at the species to assemblage level.

The most simple equation to calculate average temperature (T_{est}) from planktic foraminifer assemblage data, i.e. the ratios of species (p_i) and their optimum temperature conditions (t_i) is given by Berger (1969) as

$$T_{est} = \sum p_i t_i / \sum p_i (i = 1 \text{ to } n)$$

Imbrie and Kipp (1971) developed a conceptual ecological model of species abundance in relation to environmental parameters. The transfer function of Imbrie and Kipp (1971) includes coefficients, which account for various environmental and biological effects other than temperature, which affect the differential distribution of planktic foraminifer species:

$$P_{est} = k_0 + k_1 A + k_2 B + \cdots + k_n X \quad (10.4)$$

with P being the environmental parameter to be reconstructed, k being the empirically derived regression coefficients, and A to X being the ratios of species (of statistically significant abundance!) from census counts. The Imbrie and Kipp (1971) model applies the results of factor analysis (multivariate statistical regression) of planktic forminifer census counts, and synthetic variables characteristic of five assemblage groups (i.e. tropical, subtropical, polar, sub-polar, and 'gyre margin'), to obtain more refined temperature reconstruction (see, e.g., CLIMAP 1976). Apart from the quality of down-core census counts, the quality of transfer calculations crucially depends on the accuracy of the modern dataset compiled from surface sediment samples (e.g., Hilbrecht 1996; Pflaumann et al. 1996; Kucera et al. 2005).

New transfer methods have been developed from the classical method of Imbrie and Kipp (1971), including the Modern Analogue Techniques (MAT, Hutson 1980; and SIMMAX, Pflaumann et al. 1996), Artificial Neural Networks (ANN, e.g., Malmgren and Nordlund 1996), and the Revised Analogue Method (RAM, Waelbroeck et al. 1998) (for a review see Guiot and de Vernal 2007). All of those methods are based on modern analogue data from surface sediments. Unfortunately, the geographical and temporal coverage of data on live planktic foraminifers is too incomplete to be applied as modern analogue in transfer calculations. In addition, assemblage data from surface sediments do better represent down-core assemblages both of which having experienced alteration during sedimentation. In turn, ecological data directly derived from live planktic foraminifers (e.g.,

Lombard et al. 2011) would possibly improve the accuracy of transfer calculations.

Transfer calculations using planktic foraminifers are largely limited to the Quaternary. Modern analogues could possibly not be applied to time-intervals much older than Quaternary, since ecological demands of species, and the composition of species assemblages have evolved over geological periods of time (cf. De Vargas and Pawlowski 1998). In addition, transfer calculations are limited to the regional scale, or the scale of oceans basins at maximum, depending on the coverage of the surface (analogue) data (cf. Pflaumann et al. 1996). The regional distribution of planktic foraminifer species, i.e. morphotypes, and more importantly genotypes (e.g., Darling and Wade 2008, see Chaps. 2 and 7) with varying ecological demands, further limits the regional applicability of transfer calculations. Transfer functions are hence inherently based on simplification, since it is impossible to account for the entire complexity of abiotic and biotic parameters. However, transfer calculations may still produce non-analogue situations at the regional scale, resulting from the degree of (falsely) assumed analogy and model calibration, and so far unidentified changes of environmental and biological prerequisites over time (e.g., Guiot and de Vernal 2007).

Transfer calculations on planktic foraminifers have been applied to the Quaternary North and South Atlantic Ocean, and Indian Ocean with great success, facilitated by the good preservation of planktic foraminifer tests (e.g., Vincent and Berger 1981; Dittert et al. 1999, and references therein). In general, transfer calculations based on planktic foraminifers have been among the most valuable tools in paleoceanography over the past 40 years, and have greatly advanced our understanding of the changing oceans and climates during the Quaternary. In addition to temperature reconstruction, other parameters like primary productivity have been reconstructed with transfer functions (Ivanova et al. 2003). Like any other tool in paleoceanography, transfer functions are ideally applied in a multi-proxy approach, i.e. in combination with data on, for example, stable and radioactive isotopes, and element ratios (e.g., Fischer and Wefer 1999, and references therein; see Chap. 9).

10.14 Applications

Planktic foraminifers are widely used proxies in many fields of academic and commercial applications such as, for example, paleoceanography, biostratigraphy, and hydrocarbon exploration. Planktic foraminifer tests are ubiquitously used in paleoceanograhy, and have been reported 'intelligent design for paleocenography' (Jonathan Erez, Hebrew University of Jerusalem, oral communication), and 'paleo-argo floats' (Andy Ridgwell, UC Riverside, oral communication). The application of planktic foraminifers goes beyond the use in biostratigraphy and paleoceanography, facilitated by technological and new methodological approaches. New approaches employ planktic foraminfers for the monitoring of ecological impacts of wastewater disposal by, for example, hydrocarbon industries. In addition, test production of planktic foraminifers may provide a measure of ocean acidification, and anthropogenic impact other than CO_2 emissions. Since planktic foraminifer production is affected by, and does affect, the global carbon cycle, planktic foraminifers may indicate and mitigate environmental change on various temporal and spatial scales.

Considering biological, biogeochemical, ecological, and sedimentological processes, planktic foraminifers provide powerful tools to reconstruct ancient marine systems and climatic conditions (e.g., Vincent and Berger 1981; Shackleton 1987; Sarnthein et al. 2003; Kucera et al. 2005; Kucera 2007). In addition to the obvious use of planktic foraminifer in paleoceanographic and paleoecological analyses, planktic foraminifers provide useful proxy data in all kinds of studies of the marine carbonate system, over centennial to orbital (Milankovitch) time-scales (e.g., Rohling et al. 2012). Contributing a significant amount to the marine planktic biomass at the lower heterotrophic level (Buitenhuis et al. 2013), planktic foraminifers are actively contributing to the marine carbon

turnover, and are not only 'passive' recorders of the hydrology of ambient seawater. Considering this, planktic foraminifers may be taken into account as active mediators of the past CO_2 budget, marine carbon turnover, and for their specie-specific effects on the regional biogeochemistry and ecology. More specifically, planktic foraminifers counteract the CO_2 drawdown of the non-calcareous plankton in (iron) fertilized Southern Ocean waters (Salter et al. 2014), and register decreasing pH of ambient seawater (Ocean Acidification, OA) at the same time (de Moel et al. 2009; Moy et al. 2009).

10.14.1   First Example: Ocean Acidification (OA)

Ocean Acidification (OA) caused by increasing atmospheric and surface water CO_2 concentration potentially affects production and dissolution of planktic foraminifer tests. Calcification of modern planktic foraminifer tests has reduced by ~ 30 % compared tests from below the surface mixed sediment layer in the Arabian Sea (de Moel et al. 2009, *G. ruber*) and pre-industrial sediments south of Tasmania (Moy et al. 2009, *G. bulloides*), the latter of which were sampled from Southern Ocean waters being major sink of modern atmospheric CO_2 (Khatiwala et al. 2009). A similar negative feedback of planktic foraminifer test weight has been shown for glacial-interglacial CO_2 changes using *G. bulloides* from the temperate eastern North Atlantic, but which was affected by an additional change in calcification temperature (Barker and Elderfield 2002). However, all three studies (Barker and Elderfield 2002; de Moel et al. 2009; Moy et al. 2009) were carried out at sites of different surface marine pCO$_2$ and atmospheric CO_2 uptake of modern surface ocean waters (Khatiwala et al. 2009), and hence being source of CO_2 (Arabian Sea) or sink of atmospheric CO_2 (North Atlantic and Southern Ocean) on an annual average (Takahashi et al. 2002).

The size-normalized test weight of the symbiont bearing *G. ruber* from Arabian Sea waters shows only very slight positive relation to CO_3^{2-} concentration between 170 and 280 µmol kg^{-1} (Beer et al. 2010a). In contrast, calcification of symbiont-barren *G. bulloides* from the same water is strongly related to $[CO_3^{2-}]$ and $[CO_2]$ to the opposite direction as *G. ruber* (Beer et al. 2010a). The same CO_2-related loss in test weight and calcite production of ~ 30 % of the two species *G. ruber* and *G. bulloides*, although reported from different water masses, is hence not easy to explain. An alternative and much easier explanation of decreasing test calcite mass from the pre-industrial to modern ocean would be the dissolution of tests during sedimentation (e.g., Berger and Piper 1972; Lohmann 1995; Broecker and Clark 2001a). Dissolution of tests at decreasing $[CO_3^{2-}]$ and Ω, and increasing pH in the subsurface water column (Schiebel et al. 2007) and in surface sediments would result in weight-loss and shell-thinning of all species only depending on their dissolution susceptibility (see Dittert et al. 1999, and references therein). In addition, dissolution at deeper water bodies would be much less regional and much less affected by seasonal changes and hence more balanced than changes in calcite production in surface waters.

The effect of OA and decreasing pH on the calcite production of planktic foraminifers, and between different symbiont-barren and bearing species is not yet well understood. In case planktic foraminifers would be able to adjust to increasing CO_2 in the same way as coccolithophores by selecting for those species (clones), which are capable to sustain (or enhance) calcification (Lohbeck et al. 2012), OA might not affect planktic foraminifer calcite production at the global scale. Future planktic foraminifer calcite production might hence be even more dominated by symbiont bearing species capable of compensating for CO_2 increase (Köhler-Rink and Kühl 2005), and shift towards subtropical and tropical waters of high year-round radiation sustaining symbiont activity.

10.14.2 Second Example: Sapropel Formation

Formation of Mediterranean sapropels during anoxic events has been reconstructed in detail from planktic foraminifer population dynamics, and stable isotope analysis of major planktic foraminifer species in combination with other structural (e.g., alkenones, TEX_{86}) and chemical (e.g., Ti/Al ratio) proxies of temperature and terrestrial input (e.g., Weldeab et al. 2002; Rohling et al. 2004; Hayes et al. 2005; Castañeda et al. 2010; Hennekam et al. 2014; Mojtahid et al. 2015). Diachronous shifts of stable isotope values across the Eemian Sapropel S5, and the presence/absence of different planktic foraminifer species (Fig. 10.11) are assessed to reconstruct changes in seasonality (*Globigerinoides ruber* white and *Globigerinoides sacculifer* relative to *Neogloboquadrina incompta*), stratification of surface to subsurface water masses (*G. ruber* white relative to *G. scitula*), surface water salinity and riverine runoff ($\delta^{18}O$ of *G. ruber* white relative to *O. universa*), and trophic state of water masses ($\delta^{13}C$ of *O. universa* and *G. sacculifer*).

The multi-species planktic foraminifer study of Rohling et al. (2004) confirms significantly increased freshwater input, enhanced biological productivity, shoaling of the pycnocline, and stagnation of subsurface circulation during sapropel formation, relative to non-sapropel
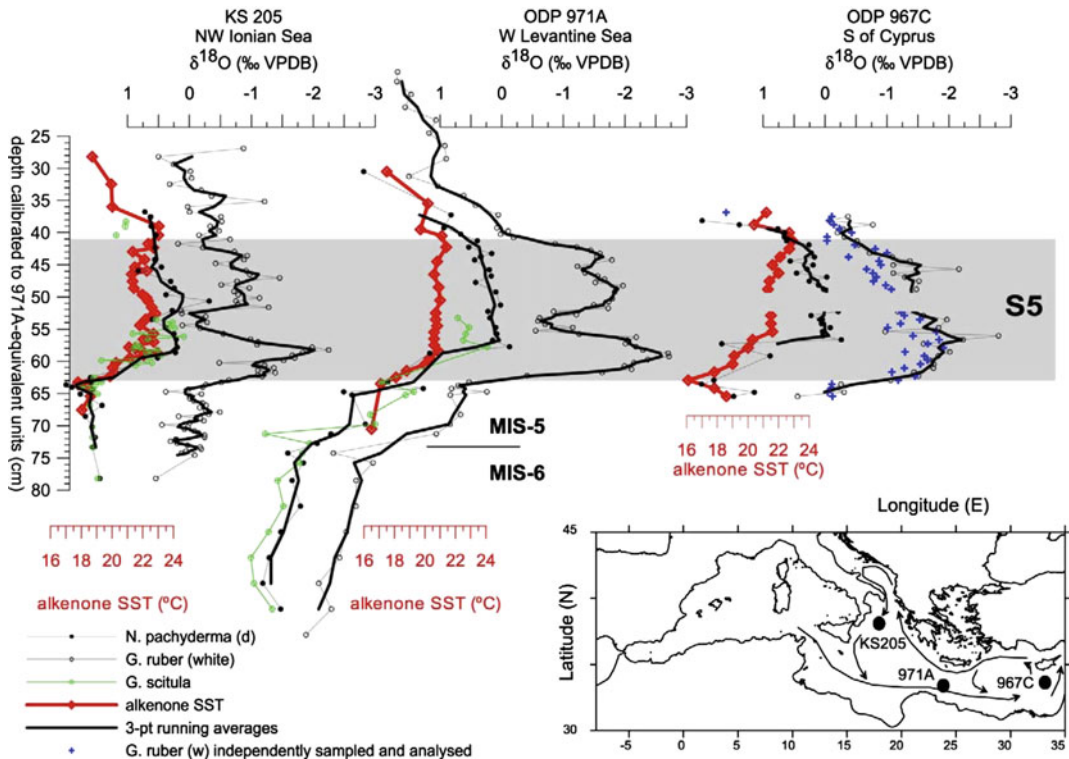
Fig. 10.11 Stable oxygen isotope and alkenone SST records of Sapropel S5 from three sites in the eastern Mediterranean. $\delta^{18}O$ of the three planktic foraminifer species *N. pachyderma*, *G. ruber*, and *G. scitula* show differential reactions to changing environmental conditions, resulting from different dwelling depth and autecological prerequisites. The subsurface-dwelling *G. scitula* (*green symbols*) disappears during S5, possibly cause by increasing oxygen deficiency in the subsurface water column. $\delta^{18}O$ values of surface-dwelling *G. ruber* (symbiont-bearing) and *N. incompta* (i.e. *N. pachyderma* d, symbiont-barren) indicate different synecological and autecological reactions, which may display differences in seasonality, ambient water temperature, salinity, and trophic conditions. The scale of SST records is adjusted to 1 °C corresponding to 0.23 ‰ on the $\delta^{18}O$ scales. From Rohling et al. (2004), and references therein

conditions in the eastern Mediterranean (cf. Rossignol-Strick et al. 1982). A similar scenario is assumed from planktic foraminifer assemblage counts and morphometric data during formation of the Holocene Sapropel S1 (Mojtahid et al. 2015). Significantly increased test sizes of both types of *G. ruber* white sensu stricto and sensu lato (see Chap. 2 Classification) during sapropel conditions indicate increased Nile River freshwater runoff, in combination with Ti/Al ratios (Hennekam et al. 2014). It is assumed that freshening of surface waters off the Nile River delta caused impaired ecological conditions, and delayed reproduction of planktic foraminifers, which led to prolonged maturity and growth of large individuals (Fig. 10.12). Finally, planktic foraminifer based proxies are applied in combination with additional chemical and structural proxies such as Sr and Nd isotope ratios, $U_{37}^{k'}$ and TEX_{86} records to achieve maximum information, and facilitate comprehensive syntheses of the paleo-environment and paleoclimate.

The two examples on Ocean Acidification and Sapropel Formation presented above in brief merely indicate to which extent foraminifers can be employed as proxies in paleoceanography, climate research, and stratigraphy. The entire application spectrum is not limited to the

chemical elements and isotopes discussed above, but includes a wide range of chemical elements and isotopes (e.g., Henderson 2002), and beyond the limits of current knowledge and feasibility. Options multiply when applying the range of methods (chemical and physical) and proxies to the different foraminifer species including morphotypes and genotypes. Moreover, certain proxies are applicable as multi-purpose tools. For example, stable oxygen isotopes yield information on the environment (e.g., temperature, salinity, and ice volume) and stratigraphy at the same time (Fig. 10.11). When adding data on population dynamics (e.g., species' abundance) and the morphometry of individual tests and entire assemblages (e.g., calcite mass, test size, and porosity; Fig. 10.12), information again multiplies. The ultrastructure and composition of the organic tissues (e.g., N isotopes; Ren et al. 2009, 2012a) of foraminifers has not yet been analyzed to its full extent, and will add another new scope to the understanding of foraminifers and their applicability. Modern technology such as LA-ICP-MS and NanoSIMS provides detailed fine-scale data, for example, on diurnal changes in calcification under varying environmental conditions (e.g., Spero et al. 2015). Finally, complementary data from non-foraminifer proxies
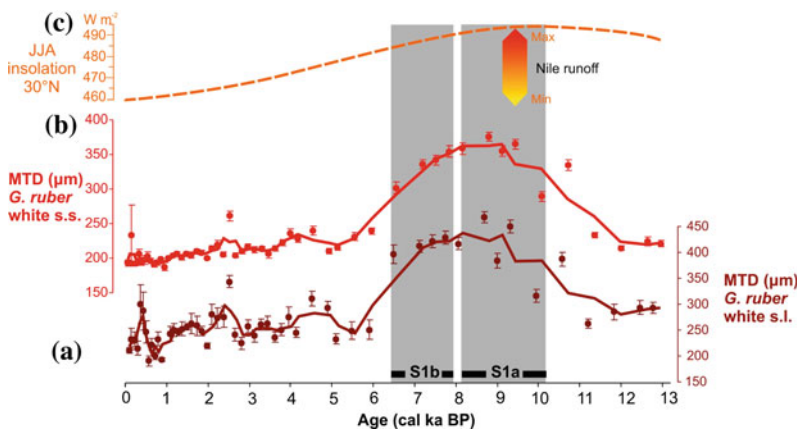

Fig. 10.12 Changes in minimum test diameter (MTD) of **a** *G. ruber* white sensu lato (s.l.) and *G. ruber* white sensu stricto (s.s.), in relation summer (June, July, August, JJA) insolation at 30°N. Summer insolation at 30°N affects Indian monsoons, precipitation at the sources of the White Nile and Blue Nile, and runoff of Nile waters into the eastern Mediterranean Sea. S1a and S1b indicate time-periods of early and late Sapropel S1 formation, respectively. After Mojtahid et al. (2015)

comprise important information (e.g., Fischer and Wefer 1999), and synergetic effects foster a better systematic understanding and quantification of processes and budgets of the changing ocean. Along with the rapid technological development, new questions and challenges will arise, and remedy may be provided. Ultimate goal of the community effort in (paleo-) environment and climate research are implementation in programs for a more sustainable management of the ocean and climate, and to preserve a habitable planet.

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