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Colonization by the benthic foraminifer *Rosalina (Tretomphalus) concinna* of Mediterranean drifting plastics

Frans J. Jorissen

CNRS UMR 6112 LPG-BIAF, Angers University, France

Abstract

Floating plastic debris, sampled with a plankton tow around Corsica, in November 2011, January and March 2014, were investigated for the presence of benthic foraminifera. The sample of November 2011 yielded 144 live specimens of *Rosalina (Tretomphalus) concinna*, corresponding to 19.3 individuals per 100 cm^2, forming a monospecific assemblage. The appearance of monospecific assemblages of *R. concinna* can be explained by the fact that this taxon is one of the very few benthic foraminiferal species which produce a planktonic stage prior to sexual reproduction. Laboratory evidence has shown that in *Rosalina* sexual reproduction occurs only at temperatures above 18°C. This would mean that around Corsica, colonization of the plastic debris can only take place in late summer and autumn. It is possible that the critical temperature limit for sexual reproduction has only been reached recently, due to climate warming. Foraminiferal colonization would be a response to multiple factors: the presence of colonizable substrates in the surface waters and a recent temperature increase. At present, it is too early to conclude what is the impact of foraminiferal colonization on the degradation of the plastics, and on the dispersal capacities of *R. concinna*.

Introduction

Since the beginning of the 21st century, there is a growing concern for the harmful effects of floating plastic debris in the marine environment (e.g., Derraik, 2002). Floating plastics endanger marine life directly by entanglement, ingestion, or absorption of the organic components. They may also impact marine ecosystems indirectly, by offering available substrates for rafting species, some of which may be exotic, or even invasive. In fact, a wide range of epibiontic marine organisms colonize floating plastics, including bryozoans, hydroids, bivalves, barnacles, tube worms, coralline algae and foraminifera (e.g., Barbes and Fraser, 2003; Barnes and Milner, 2005; Gregory, 2009; Barnes, this volume). The ecology of these rafting taxa and the substrates they colonize have been extensively reviewed by Thiel and Gutow (2005a,b). Recently, Carson *et al.* (2013b) showed that this colonization is not limited to macrofauna, but that plastics are also colonized by micro-organisms, including coccoid bacteria, centric diatoms, dinoflagellates, coccolithophores and radiolarians. Collignon *et al.* (2013) studied floating micro- and meso-plastic particles in the Bay of Calvi, Corsica, between August 2011 and August 2012. They observed that 22% of the examined particles were colonized by fouling organisms, mainly small algae and foraminifera. Based on this intriguing report, we decided to study the presence of foraminifera on floating plastics in more detail. Here, we present the preliminary results of this study.

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Material and methods

Three samples were collected in the surface waters at station BH2 off Bastia, Corsica, France, on 11 November, 2012, 8 January, 2014 and 12 March, 2014. In all three cases a Manta plankton net with a mesh size of 150 µm was applied for half an hour. In November 11, sea surface temperature (SST) was about 19°C vs. 15.2°C, in January 2014 and about 14°C in March 2014. Salinity was about 37.5 for all three sampling periods.

All samples where preserved in a mixture of 95% ethanol and 2g/l Rose Bengal (RB) stain. In the laboratory (always after more than one week of staining with RB) the samples were sieved over a 63 µm mesh, which retained all plastic fragments above this size and all adult and pre-adult foraminifera, not only those which were still attached to the plastic debris, but also those which were detached from the plastic fragments during sample transport and treatment. After this, all plastic fragments were very carefully examined on both sides under a binocular microscope. The surface of all fragments was measured (with a measurement objective in case of small pieces, with a ruler for the largest pieces), and foraminifera still attached to the plastic fragments were cut off in order to be stored (together with the plastic to which they were still attached) in Chapman slides. The remaining sieve residues, mainly containing biotic particles detached from the plastic debris during sample treatment was also studied carefully, and all RB-stained foraminifera were picked out (wet, in 50% ethanol) and stored in Chapman slides.

The Rose Bengal (RB) is a protein stain (Walton, 1952) that serves to verify whether the foraminifera were alive at the time of sampling. In the very well oxygenated surface waters of the Mediterranean, foraminiferal protoplasm (and the proteins is contains) will be degraded very quickly after the death of a foraminiferal individual, and the intense pink color of all stained foraminifera leaves no doubt that all stained individuals were alive at the time of sampling.

Results

The sample collected in November contained abundant plastic debris, of variable size (until 10 cm) and nature, whereas both samples taken in 2014 contained only some small plastic fragments (8 mm maximum length). For the sample taken in November 2012, the plastic fragments showed a large variety in faunal colonization; some fragments showed a dense cover of epibenthic (fixed) organisms, whereas other fragments were almost devoid of fauna.

Only some of the scarce fragments of both 2014 samples were colonized by sessile organisms. In view of the poverty of the 2014 samples we will base our discussion mainly on the November 2012 sample.

The November 2012 sample yielded a large amount of plastic remains, with a total estimated surface (only a single side quantified) of 749.6 cm^2. A wide variety of organisms was observed on the plastic fragments, with a strong dominance of benthic macroalgae, hydrozoans (order Leptothecata), gastropods (Nudibranchia) and bryozoans.

In the 2012 sample, a total of 144 Rose Bengal stained foraminifera were inventoried. Among these, 23 were still attached to the plastic fragments, whereas 121 were found detached from the plastic, apparently as a consequence of the sample treatment. No living foraminifera were found in the January 2014 sample, whereas 3 detached specimens were found in the March 2014 sample. All inventoried foraminifera belong to *Rosalina concinna* (Fig. 1), constituting a monospecific assemblage of this species. For the 2012 sample their density was 19 foraminifera per 100 cm^2.

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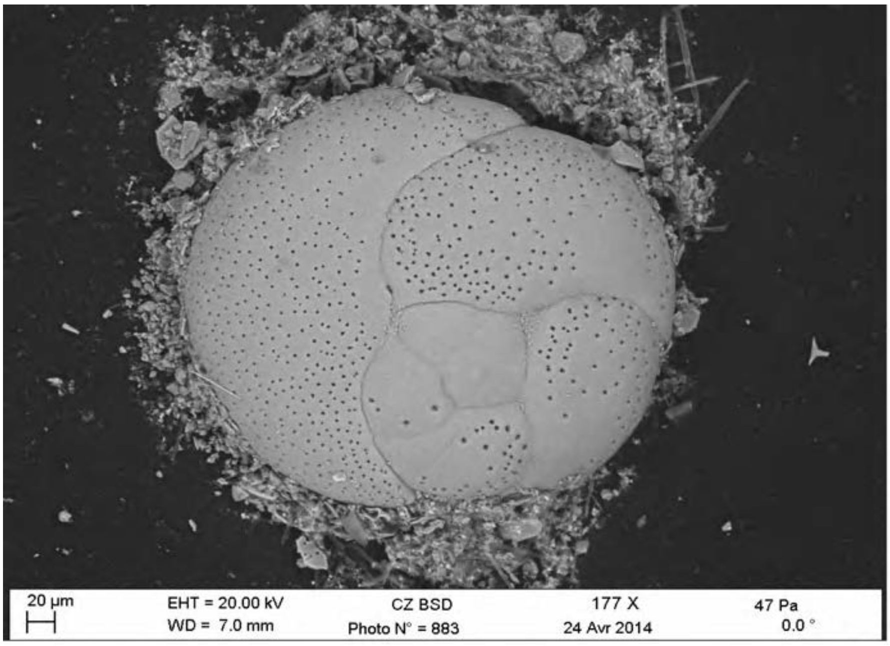

Figure 1. Adult specimen (350 µm) of *Rosalina concinna* fixed to a plastic debris. Note the organic remains concentrated around the test, which are the remnants of the feeding cyst, by which the foraminifer is fixed to its substrate.

DISCUSSION

Life cycle, ecology, taxonomical aspects and molecular phylogeny of the genus *Rosalina*

The observation that benthic foraminiferal assemblages on floating plastics are monospecific is highly surprising in view of the wide range of epiphytic taxa that have been described on macroalgae in the Mediterranean (e.g. Langer, 1993). Apparently *Rosalina concinna* is the only benthic foraminiferal species capable to colonize available substrates at the sea surface. It appears that this unique capability is due to the highly particular reproductive cycle of this species (Fig. 2). In fact, some species of the genus *Rosalina* dispose of a planktonic stage, which is a very rare treat in benthic foraminifera.

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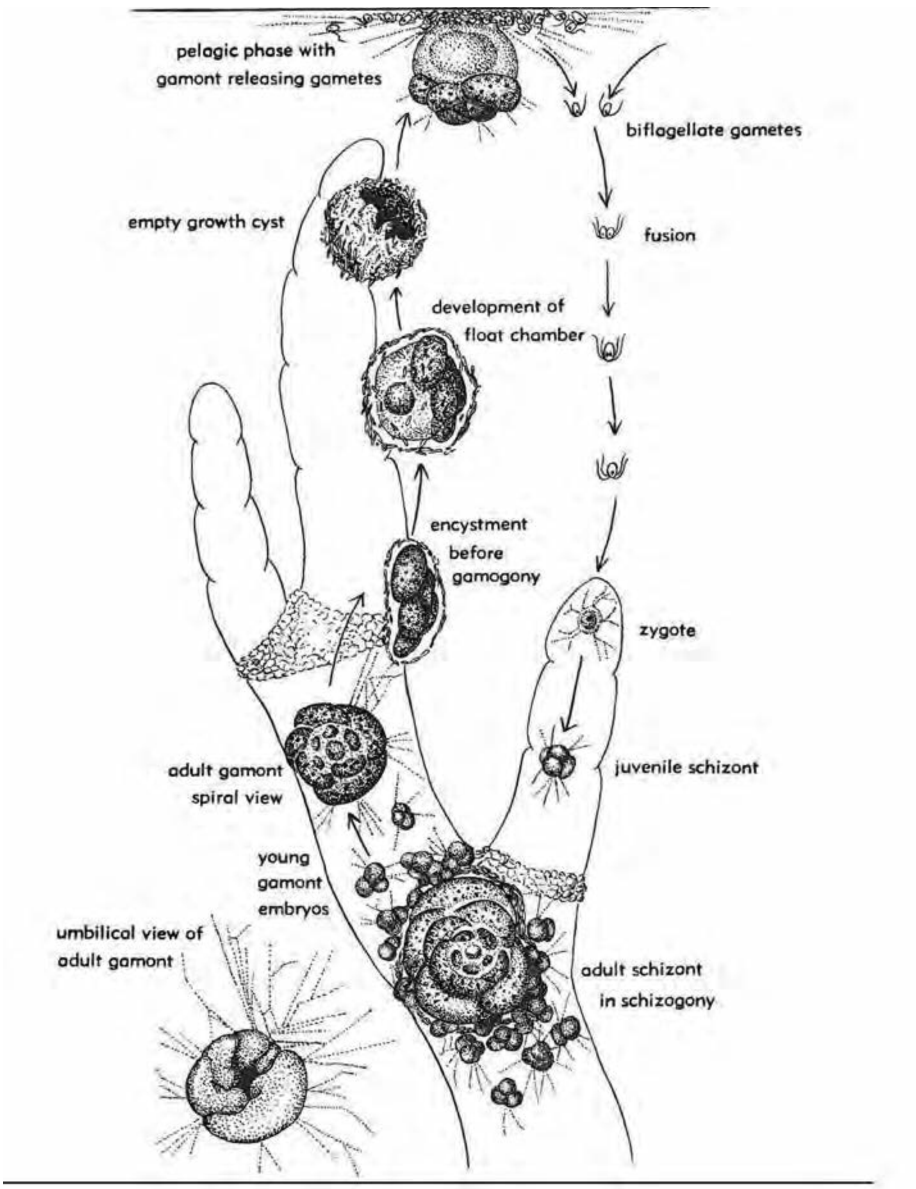

Figure 2. Life cycle of *Rosalina globularis* (Loeblich and Tappan, 1964, after Myers, 1943).

Most benthic foraminifera alternate sexual and asexual reproductive events. The schizont, characterised by a microspheric test, with a small initial chamber, reproduces asexually, whereas the gamont, with a megalospheric test (larger initial chamber), produces gametes, and reproduces sexually (Figs. 2,3). The particularity of some species of the genus *Rosalina* is that prior to producing gametes, the gamont produces a large floating chamber (the so-called *Tretomphalus*-stage), which allows it to liberate the gametes in the surface waters, optimizing their dispersal (Fig. 3).

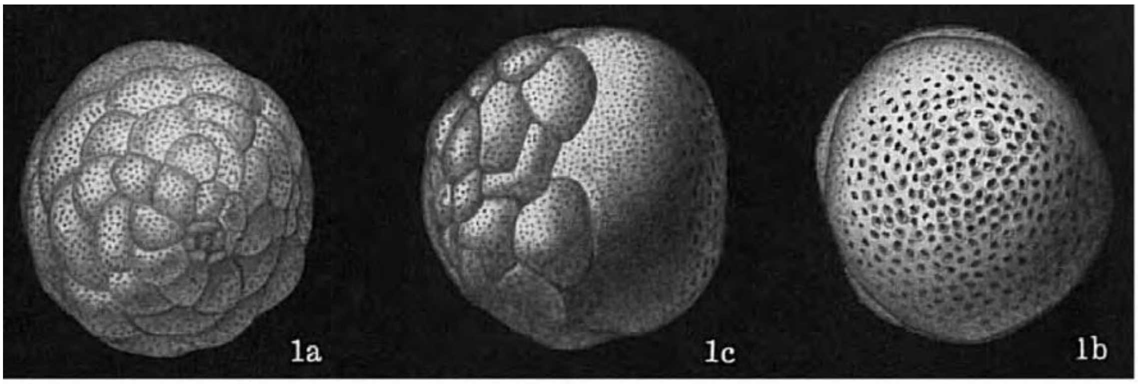

Figure 3. Floating chamber preceding sexual production (*Tretomphalus planus*, from Todd, 1965, The Foraminifera of the Tropical Pacific Collections of the "Albatross", 1899-1900. Part 4. Rotaliform families and planktonic families [End of Volume]. Bull. U.S. Nation. Mus., 161, 139 pp.)

As observed by Sliter (1965) in laboratory cultures, the strict alternation of sexual (gamogony) and asexual (schizogony) generations is not obligatory, and several successive asexual reproductions (apogamogeny) may take place if temperature is below optimal (Fig. 4). According to Myers (1943) and Sliter (1965, sexual reproduction and thus also the *Tretomphalus* floating chambers) only occurs at temperatures above 18°C. The presence of a high optimum temperature for *Rosalina globularis* was recently confirmed by Saraswat *et al.* (2011).

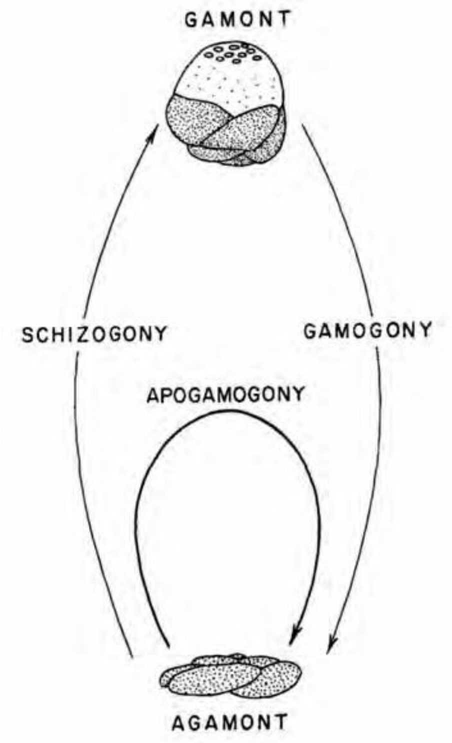

Figure 4. Life cycle of *Rosalina globularis* observed in laboratory cultures (Sliter, 1965).

In the traditional classification based on morphological criteria of the test, *Rosalina* d'Orbigny, 1826, belongs to the Discorbidae, in the order Rotaliida (Loeblich and Tappan 1964). Some authors place it in a separate family, the Rosalinidae. The planktonic stage is often considered as a separate

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genus (*Tretomphalus*, Möbius, 1880) in the same family, although other authors prefer to use it as a subgenus (e.g. Banner *et al.*, 1985). Since it has not been shown conclusively that not all species of *Rosalina* are capable top form float chambers, we follow the paper of Banner *et al.* (1985), and use the name *Tretomphalus* at a subgenus level for the species of *Rosalina,* for which float chambers have been described. In their 1985 publication, Banner *et al.* proposed the subgenus *Tretomphaloides* for forms with a *Neoconorbina*-like benthic stage, which is caracterised by strongly lunate later chambers.

Since in *Rosalina concinna*, which was chosen as type species of this new subgenus, only the last chamber is slightly lunate, this species can in our opinion not be considered as a *Neoconorbina*. Therefore, *Tretomphaloides* is here considered as a junior synonym of *Tretomphalus*.

On the basis of a phylogenetic analysis focused on the complete gene of the small subunit of ribosomal DNA (SSU rDNA), rotaliids were divided into three clades (Schweizer *et al.*, 2008). *Rosalina* was placed in a subclade together with the genera *Discorbis, Planorbulina, Planorbulinella, Rupertina* and *Hyalinea* (Schweizer *et al.*, 2008; 2009). This subdivision fairly well corresponds to the traditional morphological classification. The first three genera are all continental shelf taxa, have a planoconvex morphology, and an epiphytic lifestyle. Conversely, *Rupertina* and *Hyalinea* live in bathyal environments, on elevated habitats and as surface dwellers, respectively. Rather surprisingly, within this group planktonic stages are only known for the genus *Rosalina* (Rückert-Helbig, 1983). The molecular data available for *Tretomphalus* concern the actin gene, which has a smaller sampling range than the SSU gene (Flakowski *et al.*, 2005). *Tretomphalus* DNA sequences grouped with a specimen originally identified as *Rosalina* sp. (Flakowski *et al.*, 2005, Fig. 3)*,* but which turned out to be more closely related to *Rupertina* and *Planorbulina* in partial SSU phylogenies (Schweizer *et al.*, 2009, Fig. 6, “rotalid 3675”).

Rosalina is a cosmopolitan genus, which is largely limited to the inner continental shelf. In dead faunas the genus appears also in marginal environments, but this is probably the consequence of an offshore transport of macroalgae, to which the foraminifera are attached. In fact, *Rosalina globularis* has been described (together with *Planorbulina acervalis)* attached to *Sargassum* seaweeds sampled off Bermuda by Spindler (1980). After the decomposition of the algae, the foraminiferal tests fall to the sea floor, and may be found in much deeper environments. *Rosalina* is very common in the Mediterranean, where it is mainly represented by the species *R. bradyi* and *R. globularis*. In an environmental survey of the living benthic foraminiferal faunas of the French Mediterranean coast performed in March-April 2009, *Rosalina* spp. was a prominent taxon (20 to 150 individuals per 100 cm^2) along the southeast French coast and around Corsica (Fig. 5), where it accounts for 5 to 20% of the total foraminiferal community. These areas are characterized by sandy substrates with abundant vegetation (macroalgae and *Posidonia*-meadows). In the western part of the French Mediterranean coast, characterized my muddy substrates with scarce vegetation, *Rosalina* is almost absent.

There are two observations of the appearance of the planktonic *Tretomphalus*-stages in massive amounts. Earland (1902) reports an observation of E.H. Mathew, who found a beach deposit almost entirely composed of *Tretomphalus*-stages on Corny Point, opposite Hardwicke Bay, on York peninsula, South Australia, in November 1880. A very similar observation was made by Todd (1971), who found a beach drift at Midway atoll (in the center of the Pacific!), composed of a nearly monospecific concentrate of *Tretomphalus*-stages.

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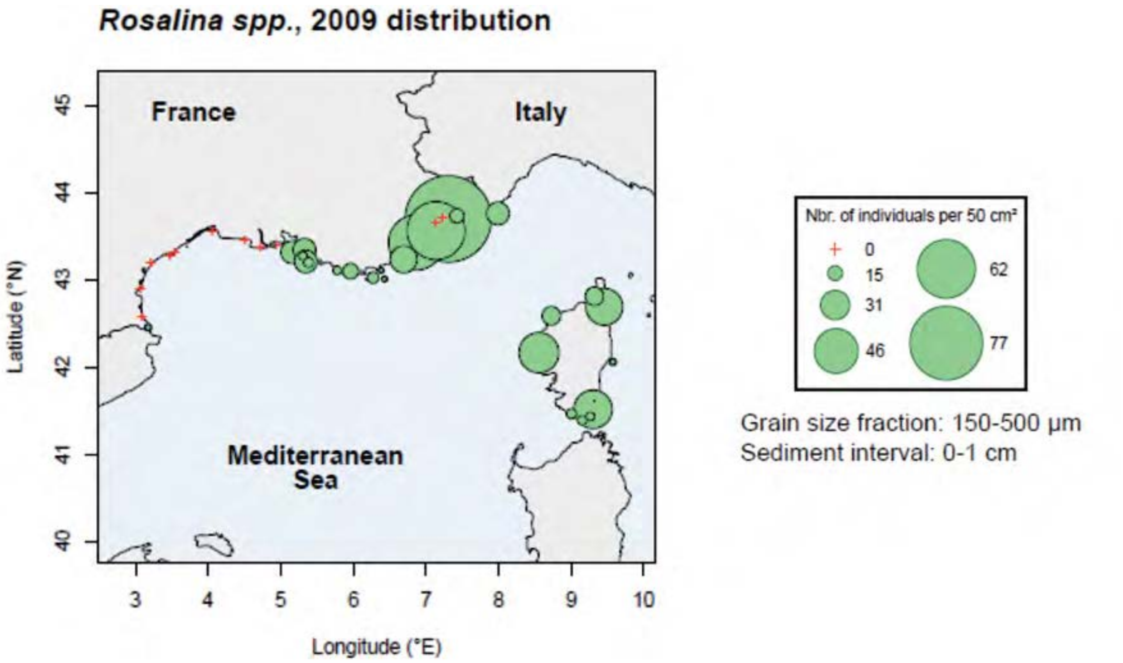

Figure 5. Distribution of *Rosalina* spp. along the French Mediterranean coast and around Corsica (Barras and Parent, pers. comm.). The circles indicate the foraminiferal density in the 150 – 500 µm size fraction of the sediment, standardized for a 50 cm^2 surface area.

In early publications on *R. globularis*, it was supposed that the European form of this species did not develop the planktonic *Tretomphalus*-stage. This idea was mainly based on the study of Vénec-Peyré (1981), who did not find any *Tretomphalus* stages in samples from Banyuls sur Mer (French Mediterranean coast), neither in the living nor in the dead assemblages. However, Tretomphalus stages have been described from the warmer eastern Mediterranean Sea (e.g. Frezza and Carboni, 2009, Northern Tyrrhenian Sea), and in a recent study, Milker and Schmiedl (2012) indicate the common occurrence of *Tretomphalus*-stages around Mallorca, offshore Oran as well as in the Alboran Sea.

Although *Rosalina* is one of the few benthic foraminiferal taxa which may develop a planktonic stage, this particularity cannot explain the very uncommon occurrence of this genus described by Fontanier *et al.* (2008). In a core sampled at 373 m depth in Saint-Tropez Canyon, which was incubated for 2.5 years with a salinity varying from 35 to 62, these authors found dense mono-specific assemblages of *R. bradyi*. In the incubated core, this species attained a density of about 500 individuals per 100 cm^2, about two orders of magnitude higher than the density found in a core sampled at the same site (3 ind/100 cm^2). The authors suggest that contrary to all other taxa, *R. globularis* survived the strongly adverse laboratory conditions, with a very high salinity.

Heinz *et al.* (2001) made a very similar observation in a feeding experiment. Surface sediment sampled at 993 m depth in the Gulf of Taranto was incubated in the laboratory for 10 months. One half of the aquarium was fed monthly with deep-frozen algae (*Chlorella* sp.). At the end of the experiment, *R. bradyi,* which is normally not found alive at this water depth, strongly dominated the faunas, in the fed (40%), as well as in the unfed (25%) part of the aquarium. The faunas were only inventoried in the upper 2.5 cm of the 15 cm deep aquarium. The density of *R. bradyi* was 1450 individuals per 100 cm^2 in the fed part of the aquarium, compared to 400 individuals per 100 cm^2 in the unfed part.

These observations suggest a highly opportunistic behavior for the genus *Rosalina*. It appears that propagules of this taxon are available even in deep sea sediments, where this species usually does not occur. After incubation *R. bradyi* is much more resistant to conditions more or less adverse than for deep-sea taxa. It survives long incubation periods, and is apparently capable to reproduce asexually, which allows it to attain very high densities.

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The densities of *R. bradyi* described in these two incubation experiments (400 to 1450 ind/100 cm^2) are at least 2 orders of magnitude higher than that observed in the field (3 ind/100 cm^2) at the 373m deep site studied by Fontanier *et al.*, 2008. The density of *R. concinna* on the floating plastics (19,3 ind/100 cm^2) is an order of magnitude lower than these spectacular values, and is similar to the minimal values found in sediment samples (Fig. 5).

However, these values cannot be compared directly. In the case of floating plastics, in most cases only a single side of the plastic is colonized, and the surface area represents the whole area available for colonization. In the case of the incubated cores or sediment samples, the standardized values represent the whole population living at and below the sediment surface, down to a depth of several cm. In view of this, the densities on the floating plastics appear to be very high.

R. concinna on plastic debris: a response to multiple stressors?

As explained in the previous chapter, it appears that *R. concinna* is the only benthic foraminiferal species capable to colonize floating plastics. Although the plastics could have been colonized by propagules, we think that the monospecific nature of the foraminiferal assemblages is a strong argument against this possibility. This monospecificity strongly contrasts with the observations made on macroalgae, on which normally fairly diverse assemblages (10 to 20 species) of epiphytic foraminifera are found (e.g., Langer, 1993). We think that the monospecific assemblages of *R. concinna* on floating plastics can only be explained by the fact that this species has a planktonic stage. Several experimental studies have indicated that in the closely related species *R. globularis*, sexual reproduction (and the formation of a floating chamber) only occurs above 18°C. Very probably, a similar lower temperature limit also exists for *R. concinna*. On the one hand, this would mean that at present, around Corsica, floating chambers can only occur in summer and autumn. On the other hand, this temperature limit could explain why the *Tretomphalus*-stage has until now not been observed on the French Mediterranean coast. It seems possible that the large scale colonization by *R. concinna* of floating plastics around Corsica is a fairly recent phenomenon, which has seen in late summer and autumn, the temperature of the surface waters rise above the critical limit for this species, allowing it to produce floating chambers and to reproduce sexually. As such, the appearance of *R. concinna* on floating plastics would be a response to the presence of floating plastic as well as a recent temperature rise.

Impact of colonization by *R. concinna* on the degradation of plastic debris

At this moment, it is not clear whether the colonization of floating plastic debris by *R. concinna* has an impact on their degradation. Generally, *R. concinna* is fixed to the plastic by means of a feeding cyst (Fig. 1), consisting of organic particles collected by the foraminifer in its surrounding environment. According to Delaca and Lipps (1972), *R. globularis* is fixed to its substrate by means of an organic membrane composed of a sulfated acid mucopolysaccharide, similar to chondroitin sulfuric acid. It has been observed that on calcareous surfaces, pits are eroded under the area covered by the foraminifera (e.g. Todd, 1965). However, Sliter (1965) considered the presence of foraminifera in small pits as fortuitous. In view of the very scant knowledge of the composition of the substance allowing the foraminifer to be fixed on its substrate, and the lack of certainty concerning the capacity of the foraminifer to bio-erode the surface it colonizes, it is not yet possible to decide whether foraminiferal colonization contributes to the degradation of floating plastic debris.

Open questions and future research perspectives

The observation of dense monospecific assemblages of the foraminifer *R. concinna* on floating plastic debris lead to in a number of research questions, which have to be addressed in future studies:

- Is the colonization of plastic debris by *R. concinna* an indirect consequence of recent global warming, which caused an increase of summer and autumn SST above this species' lower limit for sexual reproduction? A detailed study of all available literature and a comparison with historical temperature records can help answer this question.

- Are the floating plastics only colonized following sexual reproduction (formation of *Tretomphalus* floating chambers), or are the individuals which colonized the plastics also reproducing asexually in situ, thereby maintaining viable populations of benthic organisms in a surface water ecosystem ? A detailed morphological study of the early ontogenetic stages can answer this question.

• What is the impact of colonization by *R. concinna* on the degradation of the plastic debris? Do the chemical substances used by the foraminifer to attach itself, and the eventual mechanical usage, contribute to a more rapid degradation of these macro-debris? Further organic geochemical analysis of the membrane by which the foraminifera are fixed on the plastics can supply part of the answer.

• What is the impact of the colonization of plastic debris on the dispersal capacity of *R. concinna*? In this context, much depends on the dispersal patterns of the plastic debris and time involved in their degradation. However, the inferred presence of propagules of *Rosalina* even in deep-sea settings would suggest that this taxon already has an extraordinary dispersal capacity, which can hardly be increased.

Acknowledgements: I am highly indebted to François Galgani who attracted my attention on this very exciting topic, and supplied all samples. I am grateful to my Masters students Samirah Belhadj and Kévin Roger, who studied part of the material, to Christine Barras and Briz Parent, who supplied data on the recent distribution of *Rosalina* on the French Mediterranean coast, and finally, to Magali Schweizer, who briefed me on the genetic studies undertaken on the genus *Rosalina*. Gerhard Schmiedl and Yvonne Milker are thanked for the fruitful discussion on *Rosalina* in the Western Mediterranean. The SCIAM imagery facility of Angers University is acknowledged for providing the SEM picture.