1 Calcification response of planktic foraminifera to environmental change in the
2 Western Mediterranean Sea during the industrial era

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## Abstract

27 The Mediterranean Sea sustains a rich and fragile ecosystem currently threatened
28 by multiple anthropogenic impacts that include, among others, warming, pollution
29 and changes in seawater carbonate speciation associated to increasing uptake of
30 atmospheric $CO_2$. This environmental change represents a major risk for marine
31 calcifiers such as planktonic foraminifera, key components of pelagic Mediterranean
32 ecosystems and major exporters of calcium carbonate to the sea floor, thereby
33 playing a major role in the marine carbon cycle. In this study, we investigate the
34 response of planktic foraminifera calcification in the northwestern Mediterranean
35 Sea on different time scales across the industrial era. This study is based on data
36 from a 12-year-long sediment trap record retrieved in the in the Gulf of Lions and
37 seabed sediment samples from the Gulf of Lions and the promontory of Menorca.
38 Three different planktic foraminifera species were selected based on their different

39 ecology and abundance: *Globigerina bulloides*, *Neogloboquadrina incompta,* and
40 *Globorotalia truncatulinoides*. A total of 273 samples were weighted in both sediment
41 trap and seabed samples. ~~As the traditionally used sieve fractions method is~~
42 ~~considered unreliable because of the effect of morphometric parameters on the~~
43 ~~foraminifera weight, we measured area and diameter to constrain the effect of these~~
44 ~~parameters.~~
45 The results of our study show substantial different seasonal calcification patterns
46 across species: *G. bulloides* showed a slight calcification increase during the high
47 productivity period, while both *N. incompta* and *G. truncatulinoides* display a higher
48 calcification during the low productivity period. The comparison of these patterns
49 with environmental parameters revealed that Optimum Growth Conditions
50 temperature and carbonate system parameters are the most likely to influence
51 seasonal calcification in the Gulf of Lions. Interannual analysis suggest that both *G.*
52 *bulloides* and *N. incompta* did not significantly reduce their calcification between
53 1994 and 2005, while *G. truncatulinoides* exhibited a constant and pronounced
54 increase in its calcification that translated in an increase of 20% of its shell weight.
55 The comparison of these patterns with environmental data revealed that Optimum
56 Growth Conditions, Sea Surface Temperatures and carbonate system parameters
57 are the most likely parameters to influence calcification in the Gulf of Lions.
58 Finally, comparison between sediment trap data and seabed sediments allowed us
59 to assess the changes of planktic foraminifera calcification during the late Holocene,
60 including the preindustrial era. Several lines of evidence strongly indicate that
61 selective dissolution did not bias the results in any of our data sets. Our results
62 showed a clear calcification reduction between pre-industrial and post-industrial
63 Holocene and recent data with *G. truncatulinoides* experiencing the largest
64 calcification decrease (32-40%) followed by *N. incompta* (20-27%) and *G. bulloides*
65 (18-24%). Overall, our results provide evidence of clear reduction in planktic
66 foraminifera calcification in the Mediterranean most likely associated with ongoing
67 ocean acidification and consistent with previous observations in other settings of the
68 world's oceans.
69
70 **Key words:** Planktic foraminifera, foraminifera calcification, biogeochemical cycles,
71 Ocean Acidification, Mediterranean Sea.

72 ## 1. Introduction

73
74 Growing population and its linked human activity since the industrial period (defined
75 according to Sabine et al., (2004) from 1800 and therein) has caused an increase in
76 carbon dioxide, which ecological and economic consequences are considered a

77  major threat (Ipcc, 2022)1/23/23 1:04:00 PM. Atmospheric $CO_2$ concentrations
78  during the Pleistocene and Holocene ranged from 200 to 280 parts per million (ppm)
79  (Loulergue et al., 2007; Lüthi et al., 2008; Parrenin et al., 2007), but these values
80  have increased dramatically since the onset of the industrial period, exceeding the
81  threshold of 400 ppm in 2015 for the first time for at least the last 800.000 years
82  (Lüthi et al., 2008). This increase is significantly more important since the 1950s,
83  when rapid atmospheric changes due  to human activity  took place, a process
84  referred as "Great Acceleration" (Head et al., 2022a). Since then, between, 25 and
85  30% of anthropogenic $CO_2$ has been absorbed by the world's ocean (Sabine et al.,
86  2004). The ocean uptake of atmospheric $CO_2$ causes a drop in both pH and
87  carbonate ion concentration (Barker et al., 2012), lowering seawater alkalinity; this
88  process is commonly known as Ocean Acidification (OA), and it is expected to affect
89  all areas of the ocean and to have a wide impact on marine life (Hemleben et al.,
90  1989). One of the main questions about recent environmental change is how
91  different ecosystems and regions in global ocean are going to react to the ongoing
92  increase of anthropogenic atmospheric carbon dioxide.
93  A large body of evidence indicates that ocean acidification has substantial and
94  diverse effects on the distribution and fitness of a wide range of marine organisms
95  (Kroeker et al., 2013; Meier et al., 2014; Moy et al., 2009). For example, some fleshy
96  algae and diatom species have been shown to increase their growth and
97  photosynthetic activity at enhanced $CO_2$ concentrations (Kroeker et al., 2013). In
98  turn, most calcifying organisms such as calcifying algae, corals, pteropods,
99  coccolithophores and foraminifera are negatively affected by this process often
100 showing a reduction in their abundance, calcification and growth rates (Kroeker et
101 al., 2013; Orr et al., 2005).
102 Planktic foraminifera are a group of marine single-celled protozoans that produce
103 calcareous shells. Their distribution across the water column is conditioned by
104 factors that include, but are not limited to, food availability, temperature, salinity and
105 sunlight (Schiebel and Hemleben, 2005). These organisms are considered to play a
106 key role in marine carbon cycle and carbonate production, accounting for between
107 32 and 80% of the deep ocean calcite fluxes (Schiebel, 2002). Depending on their
108 ecology and feeding strategies, these organisms can be algal (dinoflagellates)
109 symbiont bearing or not symbiont bearing and be spinose or non-spinose. Planktic
110 foraminifera represent a useful tool for palaeoecological and palaeoceanographic
111 studies, as the abundances of different species and their geochemical signature
112 allow reconstructing sea surface temperatures and water column physical and
113 chemical properties (Lirer et al., 2014; Margaritelli, 2020; Schiebel and Hemleben,
114 2017).

115 Previous studies suggest that planktic foraminifera are sensitive to ocean
116 acidification (OA). Laboratory experiments indicate that when carbonate ion
117 concentration decreases, shell weight and calcification decrease too in a variety of
118 species (Bijma et al., 2002; Lombard et al., 2011). Species that host symbionts have
119 been described showing a higher tolerance to dissolution due to the capacity of algal
120 symbionts to alter immediate seawater chemistry (Lombard et al., 2009). Moy et al.
121 (2009) documented a decrease of 30-35% shell weight in the planktic foraminifera
122 *G. bulloides* during the industrial era in the subantarctic Southern Ocean, most likely
123 induced by anthropogenic-driven ocean acidification. A recent study by Fox et al.
124 (2020) showed that non-spinose (*N. dutertrei*) foraminifera species exhibit a more
125 pronounced calcification reduction than the spinose (*G. ruber*) species in response
126 to increasing $CO_2$. The main difficulty for studying the impact of OA on foraminifera
127 (and any calcifying organisms) resides in finding long-term continuous records in
128 order to be able to evaluate possible changes in shell calcification (Fox et al., 2020).
129 The Mediterranean Sea is a semi-enclosed sea with a high saturation state for calcite
130 (Álvarez et al., 2014). Moreover, the Mediterranean is also recognized as a sensitive
131 region increasing atmospheric $CO_2$ (Ziveri, 2012) due to the fast turnover time of its
132 waters (Béthoux et al., 2005) and the fast penetration of anthropogenic $CO_2$
133 (Schneider et al., 2010). Sea surface temperatures are predicted to increase by 1.5-
134 2°C by the end of the century, a faster rate than the global average (Lazzari et al.,
135 2014). pH is expected to decrease according to the global average (0.3-0.4 units by
136 2100) or even exceed the global trend (Hassoun et al., 2015). The Mediterranean
137 Sea is also affected by other stressors (Durrieu de Madron et al., 2011) which impact
138 marine organisms in many ways (Lejeusne et al., 2009). Finally, it is also a region
139 shaped by human development and its associated activities interact with
140 environmental changes (MedECC). It is often considered as a "miniature ocean" and
141 a "laboratory basin" (Bergamasco and Malanotte-Rizzoli, 2010) which makes it a
142 valuable zone to study the response of marine calcifying organisms to environmental
143 change.
144 In order to assess the impact of recent environmental change on planktic
145 foraminifera, in this work we present data from Planier sediment trap (data from 1993
146 to 2006) (Rigual-Hernández et al., 2012) and from seabed sediments from three
147 different sites located in both the Gulf of Lions and the promontory of Menorca. The
148 advantage of sediment traps is that they can provide data coming from annual fluxes,
149 avoiding the effects of seasonal abundance and ontogeny and making interannual
150 comparisons more reliable (Jonkers et al., 2019). Three different planktic
151 foraminifera species, each of which characterized by contrastingly different depth
152 habitats and ecologies, were selected for our analysis: *G. bulloides,* a spinose
153 opportunist surface dweller that lies above the thermocline (Schiebel and Hemleben,

154  2014); *N. incompta*, a non-spinose temperate surface dweller; and *G.*
155  *truncatulinoides*, a non-spinose deep dwelling species which migrates through the
156  water column with a complex life cycle. Our aims for this study are: (i) to compare
157  two widely used foraminifera weighing and size-normalization techniques and
158  provide a baseline of modern foraminifera weight data and calcification in the
159  Western Mediterranean against which future changes in foraminifera calcification
160  can be assessed (ii) document seasonal and interannual trends in the planktic
161  foraminifera calcification of the three planktic foraminifera species, and (iii) evaluate
162  possible changes in shell calcification through the Holocene to the present day by
163  comparing shell weights of the foraminifera collected by the traps with those of the
164  seafloor sediments.

## 165  2. Study area

166
167  The Mediterranean is a semi-enclosed sea and it is considered a concentration basin
168  (Bethoux et al., 1999) with a negative hydrological budget: fresh water inputs do not
169  compensate the overall basin evaporation. The surface oceanic waters that enter
170  the Mediterranean through the Strait of Gibraltar and spread towards the eastern
171  basin compensate this negative balance. ~~As described by Millot (1999), the thermo-~~
172  ~~haline properties of the inflowing Atlantic Waters (AW) are gradually modified in their~~
173  ~~transit towards the Algerian Coast in the form of the Atlantic-Ionian Stream (AIS).~~
174  ~~The AIS divides into two main branches off the western coast of Sicily: one flows~~
175  ~~south of Sicily (Béranger et al., 2004) heading towards the Eastern Mediterranean~~
176  ~~Basin, and the other one enters the Tyrrhenian Sea along the northern coast of~~
177  ~~Sicily, forming the Tyrrhenian Current (TC) (Figure 1a).~~ The Corsica Channel
178  represents the choke point for water circulation in the Northwestern Mediterranean
179  (Millot, 1999) through which waters of the Tyrrhenian flow into the Ligurian Sea,
180  where the Northern Current (NC) is formed. The NC largely controls the circulation
181  all over the western and northwestern part of the Mediterranean Sea, including the
182  Gulf of Lions (Millot, 1991) and the Balearic Sea (Fig. 1a).

183
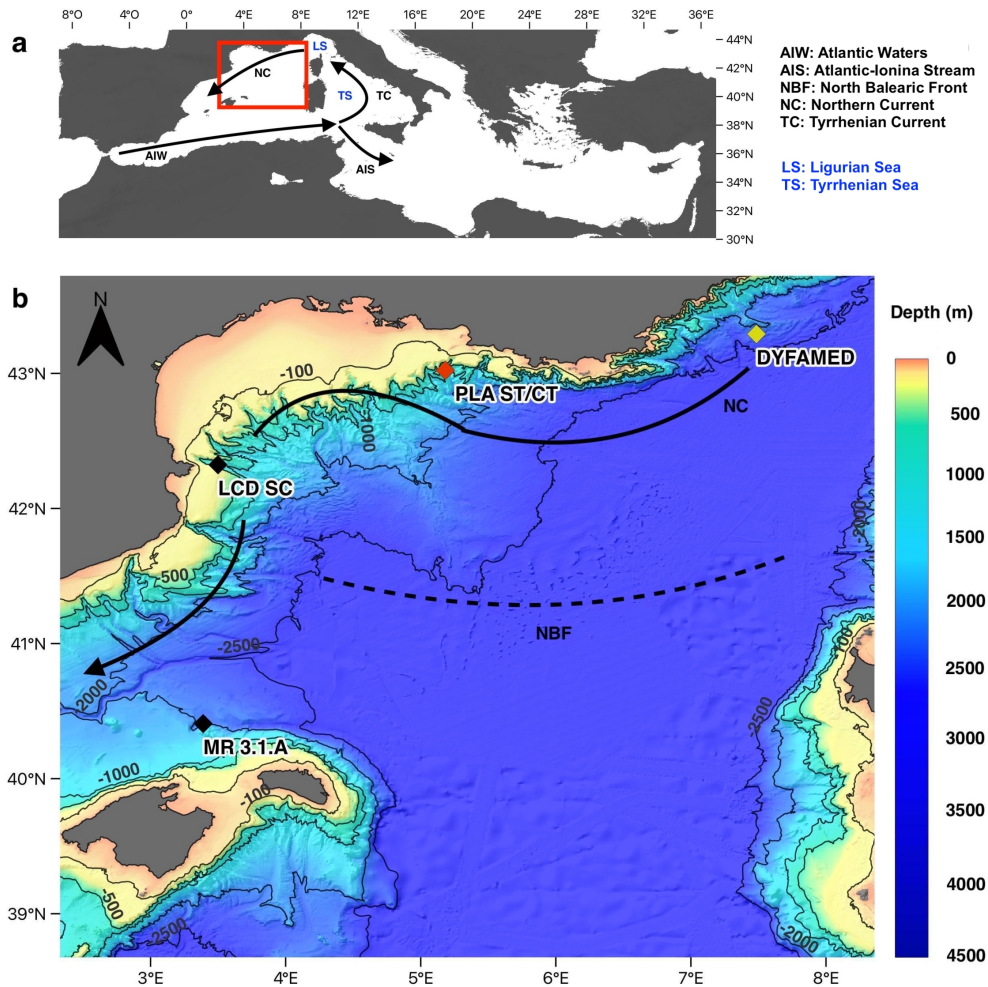
**Figure 1. a.** Study area location in the Mediterranean Sea and general surface circulation **b.** geographic setting of the Gulf of Lions and location of studied sites. Red diamond shows the position of the Planier site sediment trap and core-top (PLA ST/CT). Black diamonds represent the location of the seabed sediments samples analyzed from Lacaze Duthiers canyon (LCD SC) and Menorca promontory (MR 3.1A). Yellow diamond represents the location of the Dynamics of Atmospheric Fluxes in the MEDiterranean Sea (DYFAMED) site, located 200 km upstream Planier station position. Arrows represent the surface circulation (Millot, 1999). The topographic model was downloaded from the GEBCO database.

Moreover, the Mediterranean is recognized as a sensitive region to increasing atmospheric $CO_2$ (Ziveri, 2012) due to the fast turnover time of its waters (Béthoux et al., 2005) and the fast penetration of anthropogenic $CO_2$ (Schneider et al., 2007). Sea surface temperatures are predicted to increase by 1.5-2°C by the end of the century, a faster rate than the global average (Lazzari et al., 2014). pH is expected to decrease according to the global average (0.3-0.4 units by 2100) or even exceed

200 the global trend (Hassoun et al., 2015). The Mediterranean Sea is also affected by
201 other stressors, which impact marine organisms in many ways (Lejeusne et al.,
202 2009). Finally, it is also a region shaped by human development and its associated
203 activities interact with environmental changes (Mediterranean Experts on Climate
204 Change, MedECC, 2019).

205 The Gulf of Lions is located in the northwestern part of the Mediterranean Sea, and
206 its morphology presents a continental slope with an array of complex submarine
207 canyons (Rigual-Hernández et al., 2012) (Fig. 1b). ~~As previously stated, the general~~
208 ~~circulation is controlled by the counterclockwise NC flowing along the continental~~
209 ~~slope (Millot, 1991).~~

210 Vertical mixing, generated by intense surface cooling and evaporation, occurs in
211 winter in the Gulf of Lions driven by cold, dry northern winds, resulting in dense water
212 on the shelf and offshore (Durrieu de Madron et al., 2005; Houpert et al., 2016; Millot,
213 1991). This winter mixing recharges surface waters with nutrients. This enrichment
214 with increased solar radiation stimulates primary production in spring. Increasing
215 heat fluxes during spring and summer cause water mass stratification and nutrient
216 depletion, which lasts until late summer, until fall cooling breaks the stratification of
217 the water column and causes a fall bloom (Heussner et al., 2006; Monaco et al.,
218 1999; Rigual-Hernández et al., 2012). River inputs are the main source of suspended
219 particles in the Gulf of Lions, and the Rhone river represents the most important river
220 in the northwestern Mediterranean; however, other sources include Saharan dust
221 deposits and biological production (Heussner et al., 2006; Monaco et al., 1999).
222 Overall, the oceanographic setting of the Gulf of Lions is an exception to the general
223 oligotrophy of the Mediterranean Sea.

224 ## 3. Material and methods
225
226 ### 3.1. Sediment traps, core-tops and sediment cores.

227 A series of deployments of sediment traps mooring lines in the Gulf of Lions
228 continental margin was initiated in 1993 within the framework of several French and
229 European projects (PNEC, Euromarge-NB, MTP II-MATER, EUROSTRATAFORM)
230 and the monitoring of two sites, Planier and Lacaze-Duthiers stations (Fig. 1),
231 continues as a component of the MOOSE program (Mediterranean Ocean
232 Observing System for the Environment) (Coppola et al., 2019). Planier station
233 (43°02'N, 5°18'E) is located at the northeastern end of the Gulf of Lions, in the axis
234 of the Planier Canyon. The sediment trap used for this work was located at around
235 530 m water depth in a water column of ~1000 m. Further details of the mooring
236 design can be found in Heussner et al., (2006). Planktic foraminifera fluxes for the
237 1993 to 2006 period were documented by Rigual-Hernández et al., (2012). Here, we

used the samples from the latter study for our weight and calcification analysis. This sediment trap is used here as a baseline of the planktic foraminifera dwelling in the modern Mediterranean Sea. Moreover, we analyzed a set of core top and sediment cores collected from several locations of the Northwestern Mediterranean that are considered to represent foraminifera assemblages sedimented during the ~~pre-industrial~~ Holocene era (Table 1).

**Table 1**. Description of the core tops used in this study. Data for Planier core-top (PLA CT) and Lacaze-Duthiers sediment core (LCD SC) are available in Heussner et al., (2006), and data concerning Menorca sediment core (MR 3.1.A) can be found in Cisneros et al., (2016). Conventional $^{14}C$ ages, 1-sigma uncertainties, local reservoir and the calibrated age have been rounded according to convention.

| Site | Location | Water depth (m) | Sediment Samples | Samples Depth (cm) | Sample dated | Species dated | Radiocarbon age ($^{14}C$ years BP) | 1-sigma error ($^{14}C$ years ) | Local reservoir ($^{14}C$ years BP) | Calibrated age (cal. years BP) |
|------|----------|-----------------|------------------|--------------------|--------------|---------------|--------------------------------------|----------------------------------|--------------------------------------|----------------------------------|
| PLA CT | 42.989° N 5.121° E | 1095 | 2 | 0-1 | 0.5-1 cm | *G. bulloides* | 490 | 60 | 165 ± 95 | Out of range |
| LCD SC | 42.265°N 3.54°E | 990 | 7 | 0-5 | 0.5-1 cm | *G. bulloides* | 460 | 60 | 165 ± 95 | Out of range |
| MR 3.1.A | 40.29°N 3.37° E | 2117 | 40 | 0-27 | 14-14.5 cm | *G. bulloides* | 1980 | 65 | 165 ± 95 | 1560 |

### 3.2. Sediment core samples processing

A total of 2 sediment samples from Planier core top, 7 from Lacaze-Duthiers sediment core and 40 from Minorca sediment core were weighed (Table 1). Dry bulk sediment samples from all sites was weighed using a Sartorius CP124S balance (precision= 0.1mg).

The samples were then wet-sieved in order to separate the <63 $\mu$m fraction and dry sieved to separate the bigger fractions (>150 $\mu$m and >300 $\mu$m). The sediment washing was carried out with potassium phosphate-buffered solution (pH= 7.5) in order to optimize foraminifera preservation. Each fraction was oven dried at a constant temperature (50°C) and then weighed. The >150 $\mu$m fraction was used for identification, counting and shell morphometric and weight analyses.

### 3.3. Ecology and life cycle of *Globigerina bulloides*, *Neogloboquadrina incompta* and *Globorotalia truncatulinoides*

*G. bulloides* is a spinose surface to sub-surface dweller (Schiebel and Hemleben, 2017), found in the upper 60 m of the water column. This species has affinity for temperate to sub-polar waters and upwelling systems in lower to mid latitudes

(Azibeiro et al., 2023; Bé et al., 1977). In terms of its seasonal distribution, it has been documented associated to enhanced productivity periods in mid to high latitudes (Chapman, 2010; Schiebel and Hemleben, 2005). No symbiont algae are hosted by this species and, contrary to most spinose species, its diet is mainly algae based (Schiebel et al., 2001). *G. bulloides* shows an opportunistic feeding and strategy, leading to a high abundance in the foraminifera assemblages preserved in the sedimentary record. This is despite its tests have been documented to be more susceptible to dissolution than the average of the planktic foraminifera species (Dittert et al., 1999).

*N. incompta* is a surface dweller abundant in subpolar to temperate water masses across all the ocean basins (Kuroyanagi and Kawahata, 2004). This is a non-spinose species and does not carry symbiont algae. In North-Atlantic waters, *N. incompta* is a major component of foraminifera assemblages from late spring to late fall, and generally, is the dominant foraminifera species during late summer when maximum shoaling of mixed layer depths occur (Schiebel and Hemleben, 2000). It shows a minor presence in low latitudes and during periods of enhanced nutrient supply, *N. incompta* is outnumbered by other more opportunistic species, (Schiebel et al., 2002).

*G. truncatulinoides* is considered the deepest dweller among the extant planktic foraminifera, with living specimens documented below 2000 m (Schiebel and Hemleben, 2005). Considered a widespread species, it can be found from subpolar to subtropical water masses (Schiebel and Hemleben, 2017). It is a non-spinose species, and it does not carry any symbiont algae (Margaritelli, 2020). An important aspect to highlight about this species is its complex life cycle. reproduces once a year in the upper water column during late winter, when mixing of the water column allowed the migration of juveniles to the surface waters (Lohmann and Schweitzer, 1990; Schiebel et al., 2002). The former authors speculated that nutrient availability and the avoidance strategies to predation could explain this its life cycle. Then, the adult migrate downward the water column and spend the rest of their life cycle developing an additional calcite layer in cooler waters below the thermocline (Lohmann and Schweitzer, 1990; Wilke et al., 2009). Around 70% of *G. truncatulinoides* calcification has been estimated to take place at around the thermocline, while the remaining 30% take place in surface waters (LeGrande et al., 2004).

### 3.4. Foraminifera picking and mass and size estimations

Different sizes were selected depending on the maximum availability of each species: 250-300, 200-250 and 400-500 $\mu$m for *G. bulloides, N. incompta* and *G. truncatulinoides*, respectively. For the latter species, both coiling morphotypes were

307 selected although the right coiling was substantially less abundant representing less
308 than 3% in our counts, a feature consistent with the literature that indicates a low
309 presence of right coiled specimens (Margaritelli et al., 2020; 2022).
310 A total of 273 foraminifera samples were picked for this study, 126 coming from the
311 sediment trap and 147 from the three sediment cores and core tops (Table 2).
312 However, these numbers represent the total of samples analyzed but unique
313 samples number is lower, as not all the sediment trap samples presented the three
314 species in high enough numbers to perform the picking. The species were analyzed
315 in size fractions in order to estimate the efficiency of sieve fractions and the impact
316 of size and morphometric parameters on the foraminifera weight and calcification,
317 The mean weight of each available sediment trap sample was obtained by weighting
318 between 15 to 45 specimens of *G. bulloides* (mean N= 27), 5 to 25 *N. incompta*
319 (mean N= 15) and 5 to 25 *G. truncatulinoides* (mean N= 13). Concerning the
320 analyses of the core top and sediment core samples, between 15 and 25 *G. bulloides*
321 and *N. incompta* (mean N= 20 for both) and between 9 and 25 *G. truncatulinoides*
322 (mean N= 18) were picked.
323 Each foraminifera sample was then cleaned by gentle ultrasonication (50 Hz) for 5
324 to 75 seconds (depending on the species and the degree of visual uncleanliness) in
325 methanol in order to clean the shells. The samples were then left to dry in a
326 temperature-controlled oven at 50°C. One out of three analyzed samples were
327 weighted before and after cleaning in order to assess potential shell mass losses
328 and shell preservation due to ultrasonication. Our results indicate that this method
329 has little impact on shell preservation with around 95% of the total foraminifera
330 conserved in good conditions. Weight loss between non-cleaned samples and
331 cleaned samples are a mean 0.5 to 3 $\mu$g depending on the species, mainly due to
332 the presence of clay and non-calcite material in the shells, which justifies this
333 cleaning process (see Supplementary fig. 6).
334 The weightings were carried in the micropaleontology laboratory of the Geology
335 Department at University of Salamanca using a Sartorius ME5 balance (precision=
336 0.001 mg). This method allowed us to obtain foraminifera Sieve Based Weight
337 (SBW) by dividing the average shell weight per sample (5-45 tests) by the total
338 number of foraminifera within each sample. The lowest number of individuals
339 selected per sample was five in order to maximize the number of samples available
340 for our study. According to Beer et al., (2010), the higher the number of individuals,
341 the more reliable SBWs are. Here we aim to compare SBW results with a measured
342 weight technique. Measured techniques are acknowledged to be reliable with a lower
343 number of individuals, therefore a minimum of five individuals were selected in order
344 to compare the two techniques.

However, it has been described traditionally used sieve fractions method is considered unreliable because of the effect of morphometric parameters on the foraminifera weight (Beer et al., 2010). In order to remove the size effect on the weight, the mean SBW was normalized to the mean diameter and area of the planktic foraminifera to obtain Measurement Based Weights (MBW). Morphometric parameters were measured using a Nikon SMZ18 stereomicroscope equipped with a Nikon DS-Fi3 camera and NISElements software. These measurements were carried out on the same shells that were weighted. Foraminifera shells were positioned in order to obtain the maximum area of each individual, in this case, the umbilical side (aperture facing upwards) was measured for the three species.

$MBW_{area}$ and $MBW_{diameter}$ were calculated according to the following formula (Aldridge et al., 2012; Beer et al., 2010), where "parameter" accounts for "area" or "diameter":

$$MBW\ sample = \frac{mean\ SBW_{sample} \times mean\ parameter_{size\ fraction}}{mean\ parameter_{sample}}$$

"Size fraction" accounts for the mean of the parameter (area or diameter) measured in all the sites studied, while "sample" accounts for the mean of the parameter in the particular sample being measured. The advantage of these measurements is that the resulting MBW is being given with a weight unity (µg), thereby allowing direct comparison with other studies (Beer et al., 2010) and useful for estimating their contributions to marine biogeochemical cycles.

Correlations between SBW and $MBW_{area}$ against area are displayed in Fig. 2. The reason for this comparison is to show the relation between size and weight. In order to avoid the impact of having the bigger specimens displaying the heaviest weight and impacting the mean weight (therefore calcification indicator) of the sample.

Finally, in order to compare weights patterns from the sediment trap with weights from core tops and sediment cores and overcome the seasonality effect, MBWs were flux-weighted. Mean monthly MBWs values from each species were multiplied by the corresponding mean monthly flux and then divided by the total annual flux of the corresponding species. For these calculations, the flux data from each species estimated for the >150 µm fraction from Rigual-Hernández et al., (2012) was employed.

### 3.5. Environmental data

Foraminifera fluxes and abundances together with chlorophyll-*a* were taken from Rigual-Hernández et al., (2012) for the entire time span of the analyzed samples. Both fluxes and abundance come from direct sediment observation from the Planier

site, while chlorophyll-*a* data was obtained from SeaWiFS monthly measurements through NASA's Giovanni program on a 0.2 x 0.2° area around the mooring location. SeaWiFS measurements started in 1997 and were used due to the lack of direct chlorophyll measurements in our samples. Sea Surface Temperature (SST) was recovered from the NOAA database with the same gridding as the data from the NASA's Giovanni program.

Salinity, nutrient concentrations (nitrates and phosphates) and carbonate system parameters data were collected from the DYFAMED database (http://www.obs-vlfr.fr/dyfBase/index.php) (Coppola et al., 2008; 2021). DYFAMED site is located around 200-220 km (Fig. 1b) east of the sediment trap location (43°25´N, 7°52´E), in the Ligurian Sea. From an oceanographic view, its situation is upstream of the NC circulation and can be considered representative of seasonal and interannual variability of biological and water column properties of the open-ocean waters in the northwestern Mediterranean (Heussner et al., 2006; Meier et al., 2014). Alkalinity and total carbon measurements were available for years 1998 to 2000 and mid 2003 to 2005. Missing values comprised in these years were replaced with values obtained from linear regression of the measurements from above and below. The CO2SYS macro has been used to reconstruct the $[CO_2]$, $[CO_3^{2-}]$, $[HCO_3^-]$ and pH values from the measured total alkalinity and dissolved inorganic carbon. The constants used were the $CO_2$ dissociation constant by Mehrbach et al., (1973) refit by Dickson and Millero, (1987); the KHSO4 by Dickson, (1990); and the seawater scale for pH. In order to have uninterrupted monthly environmental values from the DYFAMED site during available measurements, a resampling every 10 days has been carried out with the QAnalySeries program.

Seasonal correlation analyses were carried out with the Statistica program. A p<0.05 was used in order to consider a correlation as significant. The number (N) of correlations depended on data availability and was 10 for *G. bulloides*, 9 for *N. incompta* and 12 for *G. truncatulinoides*. On an interannual time scale we acknowledge that our data presents some gaps over the years, and some years in the sediment trap and foraminifera record are not well represented, however, trends provided here give a new insight on interannual calcification trends in the northwestern Mediterranean.

**3.6. Statistical analysis**

In order to have uninterrupted monthly environmental values from the DYFAMED site during available measurements, a resampling every 10 days has been carried out with the QAnalySeries program.

Independence and correlation between the area the different species SBWs and MBW$_{area}$ was tested using a Pearson linear correlation test with an R script (see Supplementary material).

Seasonal correlation analyses were carried out with the Statistica program. A p<0.05 was used in order to consider a correlation as significant. The number (N) of correlations depended on data availability and was 10 for *G. bulloides*, 9 for *N. incompta* and 12 for *G. truncatulinoides*.

It should be noted that the analysis of interannual trends was hindered by gaps in the sediment trap record and by the low number of specimens during some sampling intervals. Therefore, interannual trends in planktonic foraminifera calcification should be interpreted with caution.

The influence of a suite of environmental variables upon MBW$_{area}$ was assessed using General Additive Models (GAM) (*mgcv* R-package). For each species, MBW was modelled as a smooth function of each environmental variable (see Supplementary figs. 3, 4 and 5). The limited number of data points restricted GAM complexity to a single explanatory variable, so environmental interaction effects were not assessed.

In order to investigate the difference between the MBW data from the sediment trap and the core-top /sediment cores, a non-parametric two-way Mann-Whitney test has been performed. The aim of this test is to analyze the difference between the median of the different datasets. A p-value <0.05 has been used to consider the median of two datasets different.

### 3.7. Radiocarbon dating

Between 50-100 individuals of well-preserved *G. bulloides* shells (>150 $\mu$m) were picked for radiocarbon analyses. Radiocarbon ($^{14}$C/$^{12}$C) was measured as $CO_2$ with a gas ion source in a Mini Carbon Dating System (MICADAS) at the Laboratory of Ion Beam Physics from ETH Zürich. The employed automated method consists of initial leaching of the outer shell to remove surface material with 100 $\mu$l of ultrapure HCl (0.02M) and the subsequent acid digestion of the remaining carbonates with 100 $\mu$l of ultrapure $H_3PO_4$ (85%) (Wacker et al., 2013). Therefore, no cleaning was applied after the picking contrary to the samples used for mass and size measurements. Marble (IAEA-C1) was used for blank correction and results were corrected for isotopic fractionation via $^{13}$C/$^{12}$C isotopic ratios.

Conventional radiocarbon age for sample 14-14.5cm from MR 3.1.A site was calibrated with the on-line calibration program CALIB (Stuiver and Reimer, 1993) using the Marine20 curve, which applied a marine reservoir correction of 550 $^{14}$C years (Heaton et al., 2020) to the corresponding $^{14}$C age and error. Additionally, a local reservoir effect (Stuiver and Braziunas, 1993) of -165 ± 95 $^{14}$C years was considered. This local reservoir was calculated as the average of the 8 nearest points to the sample location from the Marine Reservoir Correction database (Reimer and Reimer, 2001), whose values have already been corrected for the

461    Marine20 curve. $^{14}C$ ages from samples 0.5-1cm from both PLA CT and LCD SC
462    lied out of the range for calendar calibration, implying these samples contain some
463    bomb $^{14}C$ and cannot be considered pre-industrial. In order to have an estimation of
464    the time span that could be covered by these dates, the same marine and local
465    reservoir corrections were applied to the most recent $^{14}C$ date that could be corrected
466    (i.e. 603 $^{14}C$ years BP). As the $F^{14}C$ for this sample was <1 (see Supplementary
467    table 1), this means that the $^{14}C$ found in these samples is not dominated by the
468    bomb carbon. Here we propose a 110-50 cal. years BP age for these samples. Then,
469    these samples are considered post-industrial. The detailed results of the calibration
470    and the $^{14}C$ dating can be found in the Supplementary figs. 1 and 2.
471    Finally, it is important to consider that these $^{14}C$ ages represent mean average
472    values. Therefore, time integration within each sample and the effects of bioturbation
473    could cause a variation on the foraminifera real ages (Dolman et al., 2021).
474    Both the samples and dates obtained are detailed in Table 1. Planktic foraminifera
475    present in the dated samples that were not selected for radiocarbon dating were also
476    analyzed following the methodology described previously.

477    ## 4. Results

478

479    **4.1. Shell morphometric parameters and shell-weight normalization**
480    Table 2 shows the results of the shell area, diameter and SBW, the total foraminifera
481    samples analyzed, the mean number of individuals per sample and the total
482    individuals measured at each of the studied sites.

483    **Table 2**. Minimum, mean, maximum and standard deviation values of shell
484    area, diameter and SBW for *G. bulloides, N. incompta* and *G. truncatulinoides*
485    at all studied sites. The last 3 columns show the number of samples, the mean
486    number (N) of individuals analyzed per sample and total number of individuals
487    measured for each site.

| PLA Sediment Trap | Area (µm²) | | | | Diameter (µm) | | | | Sieve Based Weight (SBW, µg) | | | | Total samples | N per sample | total N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Min | Mean | Max | Std.Dev | Min | Mean | Max | Std.Dev | Min | Mean | Max | Std.Dev | | | |
| *G. bulloides* | 16978 | 57353 | 168492 | 17261 | 147.0 | 267.5 | 463.2 | 38.6 | 3.21 | 4.43 | 5.60 | 0.66 | 35 | 27.2 | 893 |
| *N. incompta* | 26234 | 42821 | 135422 | 8934 | 182.8 | 232.4 | 415.2 | 22.6 | 3.17 | 4.45 | 5.40 | 0.59 | 32 | 15.0 | 455 |
| *G. truncatulinoides* | 70712 | 178952 | 527622 | 63572 | 291.9 | 468.5 | 819.6 | 81.9 | 10.67 | 23.11 | 39.57 | 7.79 | 59 | 13.0 | 729 |
| **PLA Core-Top** | | | | | | | | | | | | | | | |
| *G. bulloides* | 37163 | 55395 | 87894 | 12302 | 217.5 | 264.0 | 334.5 | 28.8 | 5.00 | 5.22 | 5.43 | 0.30 | 2 | 17.3 | 39 |
| *N. incompta* | 27635 | 36927 | 49619 | 5447 | 187.6 | 216.3 | 251.4 | 15.9 | 4.46 | 4.46 | 4.46 | 0.00 | 2 | 19.7 | 41 |
| *G. truncatulinoides* | 89778 | 174748 | 233229 | 44313 | 338.1 | 467.7 | 544.9 | 61.9 | 34.80 | 35.40 | 35.90 | 0.70 | 2 | 14.7 | 34 |
| **MIN Sediment core** | | | | | | | | | | | | | | | |
| *G. bulloides* | 20895 | 52132 | 138424 | 8722 | 163.1 | 256.8 | 419.8 | 20.5 | 4.00 | 5.07 | 6.57 | 0.46 | 40 | 19.6 | 761 |
| *N. incompta* | 24003 | 35098 | 57264 | 4658 | 174.8 | 211.0 | 270.0 | 13.7 | 3.45 | 4.11 | 5.00 | 0.34 | 40 | 20.3 | 791 |
| *G. truncatulinoides* | 116686 | 166318 | 365851 | 23262 | 385.4 | 459.1 | 682.5 | 30.8 | 28.33 | 34.99 | 42.60 | 3.25 | 40 | 14.4 | 576 |
| **LCD Sediment core** | | | | | | | | | | | | | | | |
| *G. bulloides* | 27624 | 52472 | 116605 | 8793 | 187.5 | 257.7 | 385.3 | 20.4 | 4.35 | 4.73 | 5.19 | 0.31 | 7 | 20.1 | 136 |
| *N. incompta* | 28089 | 37789 | 51284 | 4972 | 189.1 | 218.9 | 255.5 | 14.4 | 3.68 | 4.12 | 4.50 | 0.26 | 7 | 19.8 | 134 |
| *G. truncatulinoides* | 82534 | 143138 | 393754 | 41620 | 324.2 | 423.3 | 708.1 | 55.9 | 25.27 | 26.68 | 30.66 | 1.94 | 7 | 15.3 | 105 |

Overall, the mean values for both diameter and area correspond to mean narrowed size fraction used during the picking, but morphometric parameters show some variability between the studied sites. Standard deviation of both area and diameter values for the three species are higher in the sediment trap record than in seafloor sediments, with mean values (of all three species) of 82% higher for area and 69% higher for diameter. SBW exhibits the same pattern as both area and diameter standard deviation is a mean 130% higher in the Planier sediment trap. Regarding the variability across the seafloor samples, Planier core-top exhibits a greater area and diameter values (about 40 to 50% increase for the three species) compared to those of the other two sediment cores, probably due to the fewer samples analyzed (Table 2).

The Planier sediment trap results (Table 2) show a higher standard deviation for both area and diameter for the three species, i.e. 76 % and 68% higher for *G. bulloides* compared to the data from core tops, 78% and 54% for *N. incompta* and 81% and 73% for *G. truncatulinoides*.

Because of the lack of precision of the initial individuals picking, carried out with a micrometer installed in the microscope, the selection is not totally accurate. Due to this issue, one third of the of the total measured foraminifera (i.e. 1645 of 4694) were out of the desired size fraction, of which 12% were bigger (580/4694) and 23% were smaller (1065/4694). Nonetheless, only 0.02% were more than 20% out of the selected size range (64/4694 more than 20% bigger and 29/4694 more than 20% smaller). Mean size difference for the foraminifera out of the size fraction is around 7%. Results vary according to the site and the species. 50% of the individuals from the Planier sediment trap (1046/2077) and 26% of the individuals coming from the

513   core tops (692/2617) were out of range. *G. bulloides* showed a 45.5% (53.2% in the
514   sediment trap and 39.3% in the core-tops samples) of individuals out of selected size
515   fraction, while this value is 21.5% (22.2% in sediment trap, 21.1% in sediment cores)
516   for *N. incompta* and 35% for *G. truncatulinoides* (53.4% in sediment trap, 16.7% in
517   sediment cores).
518
519   Even though a narrow size class was selected for each species (see section 3.4), a
520   clear influence of the area on the SBW was found in our data set (Fig. 2).
521

522
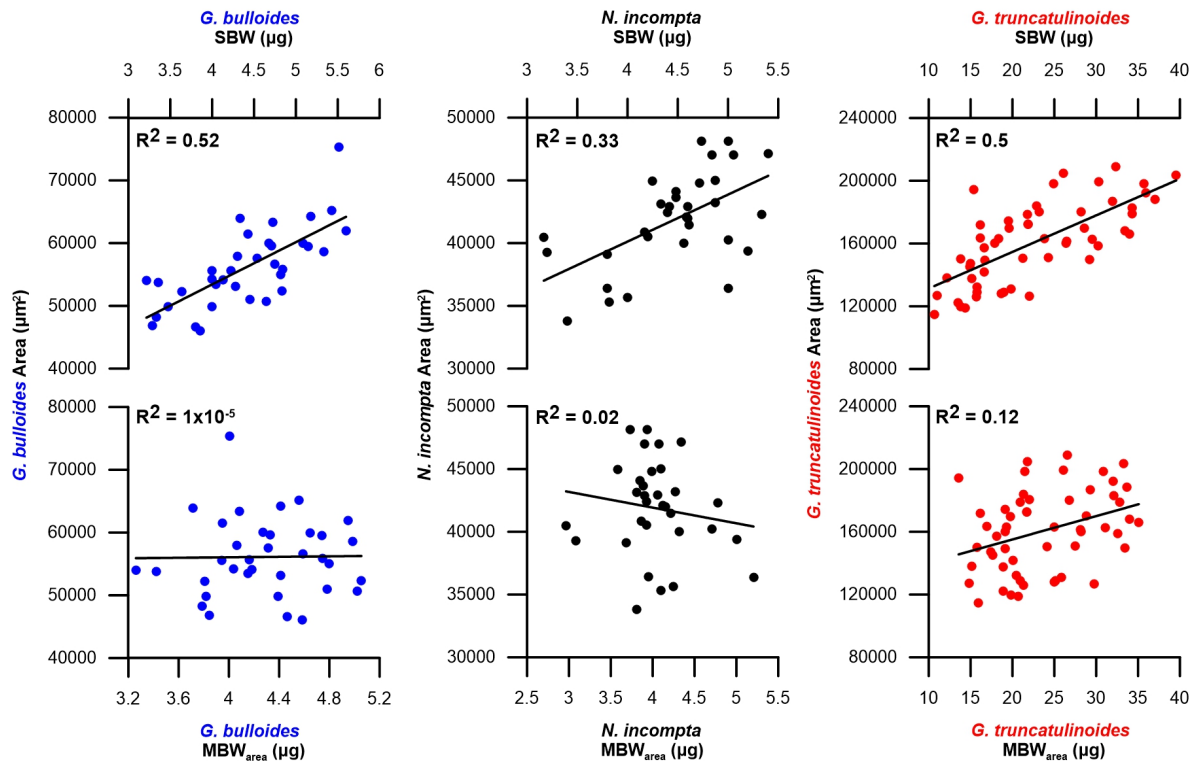
**Figure 2**. SBW in *µ*g and MBW$_{area}$ in *µ*g against the mean test area in *µ*m$^2$ for
foraminifera samples in the Planier sediment trap. Dark blue dots correspond
to *G. bulloides*, black dots *to N. incompta* and red dots to *G. truncatulinoides*.

526   In Fig. 2, we show the values for the Planier sediment trap record for both SBW and
527   MBW$_{area}$ for all three species considered in this study, and their r$^2$ with the measured
528   area. In particular, SBW shows a positive correlation with area: $0.33 < r^2 < 0.53$. This
529   indicates that the SBW is dependent on the size of the specimens within the selected
530   size range. Thus, to isolate the component of variation in foraminifera shell thickness
531   that represents a change in calcification and does not occur as a direct result of
532   changes in shell size, normalization of the shell weight was performed following the
533   formula detailed in section 3.3. (Beer et al., 2010). After normalization MBW$_{area}$
534   shows no correlations with area: $1 \times 10^{-5} < r^2 < 0.12$ (Fig. 2). Note that the weight

535  variations in our dataset are quite considerable, especially for *G. truncatulinoides*,
536  probably due to the wider size fraction. Diameter does show correlation with SBW:
537  $0.33 < r^2 < 0.5$; and shows a non-negligible correlation with $MBW_{diam}$: $0.2 < r^2 < 033$. Our
538  data demonstrates that SBW correlates more strongly with $MBW_{diam}$ than with
539  $MBW_{area}$ for the 3 species: 0.9>0.48 for *G. bulloides*, 0.89>0.52 for *N. incompta* and
540  0.97>0.81 for *G. truncatulinoides*. These values are consistent with previous studies
541  (Beer et al., 2010).

542  **Table 3.** Pearson correlation test results for the three species correlation
543  between area ($\mu m^2$) and both SBW ($\mu g$) and $MBW_{area}$ ($\mu g$). Here c.i. stands
544  for "confidence interval". Significant r values (0<c.i.<1) are set in bold.

| | Area ($\mu m^2$) | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | *G. bulloides* | | *N. incompta* | | *G. truncatulinoides* | |
| | r | c.i. | r | c.i. | r | c.i. |
| SBW ($\mu g$) | **0.72** | 0.52, 0.85 | **0.57** | 0.28, 0.77 | **0.62** | 0.41, 0.76 |
| $MBW_{area}$ ($\mu g$) | 0.014 | -0.32, 0.35 | -0.15 | -0.47, 0.21 | 0.21 | -0.09, 0.44 |

545

546  Furthermore, a Pearson correlation test (see section 3.6) has been carried out in
547  order to assess the influence of area on SBW and $MBW_{area}$ (Table 3). Results
548  showed that the SBWs from the three species correlated positively and significantly
549  (0< c.i <1). with their corresponding areas (0.57< r <0.72). Concerning the MBWs,
550  no significant (0> c.i. >1) correlations with the area are observed (-0.15< r <0.2).
551  Therefore, these correlations further highlight the fact that SBW values are
552  significantly influenced by shell area, while $MBW_{area}$ values appeared to be
553  independent of the area.
554  Differences between SBW and both $MBW_{area}$ ~~and $MBW_{diam}$~~ vary depending on the
555  species: SBW is slightly heavier for *G. bulloides*, heavier for *N. incompta* and lighter
556  for *G. truncatulinoides*. The mean standard deviation for all 3 species is around 8%:
557  7.8% for *G. bulloides*, 6.4% for *N. incompta* and 13% for *G. truncatulinoides*. We
558  take these values as the error adjustment for SBW in the different size fractions (250-
559  300 $\mu$m, 200-250 $\mu$m and 400-500 $\mu$m respectively). It is difficult to compare these
560  results with other studies as size fractions and species are often different, but this
561  error estimates are in the same order of magnitude as some other MBW published
562  in core-tops records and sediment traps (de Moel et al., 2009; Moy et al., 2009).
563  These findings highlight the fact that the use of sieve fractions does not provide
564  enough control on the influence of morphometric parameters in test weight.
565  Morphometric variations described in table 1 indicate that the typically used sieve
566  fractions may be unreliable due to the number of individuals out of the desired
567  fractions and the variability within the size range. The correlations between SBW

568 and shell area are consistent with previous studies (Aldridge et al., 2012; Beer et al.,
569 2010) and underscore the importance of isolating the component of variation in
570 foraminifera shell thickness that represents a change in calcification and does not
571 occur as a direct result of change in shell size. Thus, the shell weight was size-
572 normalized after Beer et al., (2010) by isolating the influence of isometric scaling on
573 wall thickness and calcification density.
574 Area and diameter show a perfect correlation ($r^2$= 1) for all 3 species, highlighting a
575 relationship between the different MBWs. Moreover, both $MBW_{area}$ (Fig. 2) and
576 $MBW_{diam}$, in either the sediment trap data and core-top data, do not correlate with
577 area and diameter ($1x10^{-5} < r^2 < 0.33$ and $0.001 < r^2 < 0.2$ respectively) indicating that
578 size does not have an influence on these values. This suggests that our size-
579 normalization procedure adequately removes the size influence (Fig. 2) and
580 therefore, our MBW data represents a robust parameter reflecting test wall thickness
581 and calcification intensity not influenced by test size (Table 3). Therefore, MBWs can
582 be considered as a reliable calcification intensity proxy.
583 Based on all the above, from this point we'll focus our discussion on the $MBW_{area}$
584 and ρA (area density) to discuss the foraminifera shell weight variability on seasonal,
585 interannual and pre to post-industrial Holocene time scales.
586
587

**4.2. Seasonal variations of foraminifera calcification in the NW Mediterranean**

Mean annual MBW$_{area}$ and $\rho$A values were calculated for the three species to illustrate the seasonal variability of these parameters (Figure 3). Monthly data corresponds to the average result of all the data corresponding to each month between the twelve-year time series (1993-2006) at both sites.

MBW$_{area}$ values were calculated for the three species to illustrate the seasonal variability of these parameters (Fig. 3). Samples have been assigned to their corresponding month according to the mean cup sampling date.

596
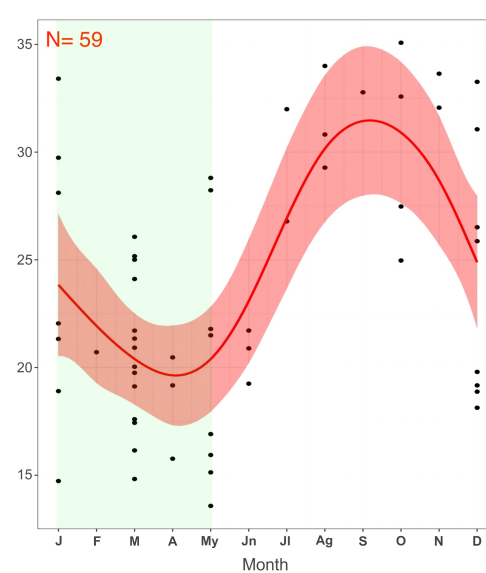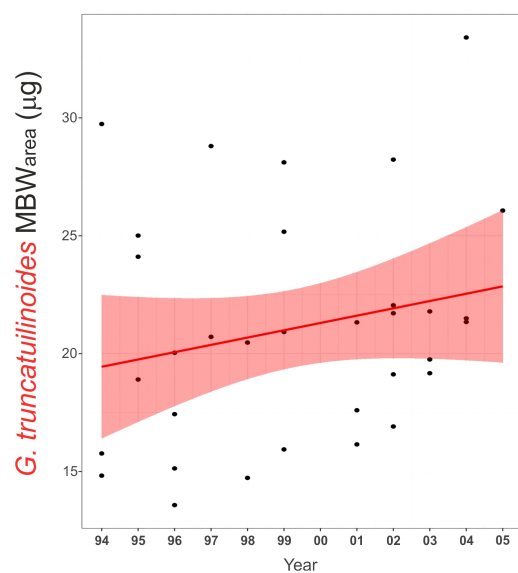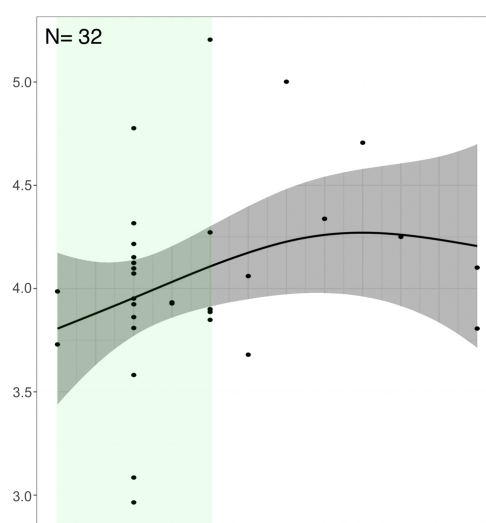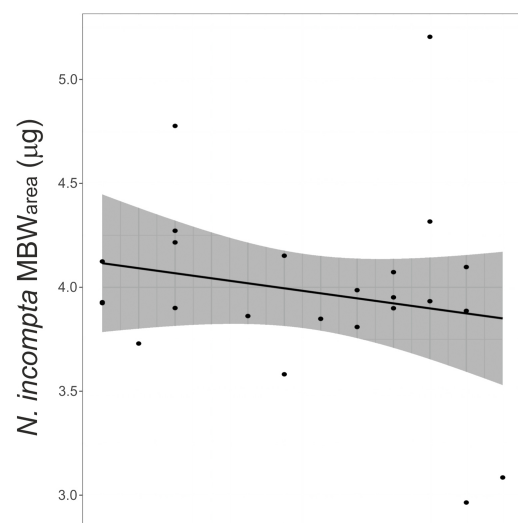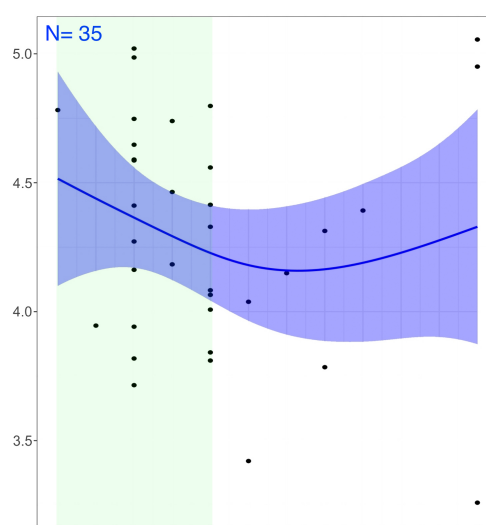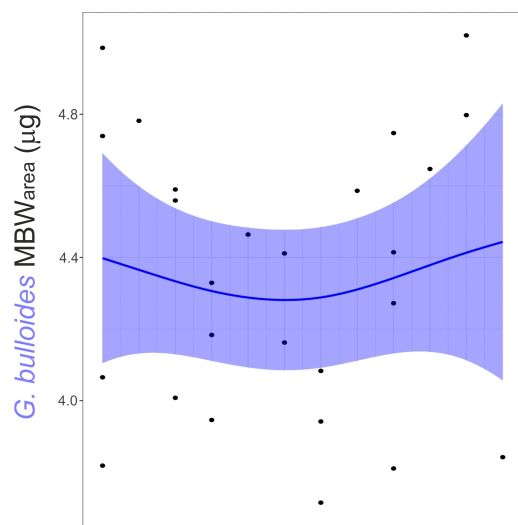
**Figure 3.** ~~Mean~~ MBW$_{area}$ (*μ*g ~~) and ρA (×10$^{-5}$)~~ values across the years and for a composite year for *G. bulloides, N. incompta* and *G. truncatulinoides* in the Planier sediment trap. Light-green shaded area represents the high productivity period in the study zone (Rigual-Hernández et al., 2012).

The mean MBW$_{area}$ for the three species in the Planier sediment trap are 4.29 *μ*g (± 0.45 *μ*g for *G. bulloides*, 4.04 *μ*g (± 0.4 *μ*g) for *N. incompta* and 23.25 *μ*g (± 6.2 *μ*g) for *G. truncatulinoides*.

~~The temporal variations in shell calcification exhibit a clear seasonal pattern for the three species. The composite year shows that all the three species exhibit a clear but different seasonal pattern (Figure 3).~~ The seasonal variations in shell calcification differ according to the species.

In the case of *G. bulloides*, maximum annual calcification values are reached during winter and early spring: 5.05 in December and 5.02 *μ*g in March. January displays the highest mean value: 4.78 *μ*g. Minimum values are reached during summer: 3.72 *μ*g in June, which is also the month that exhibits the lowest mean MBW$_{area}$. Overall, there is a 1 *μ*g seasonal difference in calcification between maximum and minimum values, which corresponds to a 24.5% change in the mean MBW$_{area}$ value. Mean seasonal standard deviation is ± 0.47 *μ*g. ~~with the lowest value in April, ± 0.27 *μ*g; and the highest value in December, ± 1 *μ*g.~~

*N. incompta* shows a maximum in calcification in late spring to mid-summer: a maximum value of 5 *μ*g is reached in May, while July is the month that displays the highest mean value (5 *μ*g). Lowest values are reached in early spring: 2.96 *μ*g in March, while January displays the lowest mean value (3.85 *μ*g). Thus, the annual mean seasonal amplitude is 1.15 *μ*g which translates into a 28% seasonal MBW$_{area}$ variability. Standard deviation is ± 0.28 *μ*g. ~~with the highest value in May, ± 0.53 *μ*g; and the lowest one in April with no deviation.~~

Finally, *G. truncatulinoides* displays a seasonal maximum MBW$_{area}$ value in late summer-autumn, with a maximum reached in October: 35.07 *μ*g, while November; is the month that shows the highest mean MBW$_{area}$ value (32.85 *μ*g). The lowest value is reached in March: 13.57 *μ*g, and April is the month that shows the lowest mean value: 18.45 *μ*g. Seasonal MBW$_{area}$ difference is 14.3 *μ*g: a 60% variability. Mean typical seasonal deviation is ± 3.7 *μ*g. ~~with December and November showing the highest and lowest variability respectively: ± 1.1 and ± 6.7 *μ*g.~~

**4.3. Interannual MBW$_{area}$ trends**

Trends throughout the 12-year record are represented in Fig 3. In order to obtain representative data for each year, maximize data availability of each species and avoid the impact of months with insufficient specimens on the interannual trends,

only MBWs from the productive period (January to May) of each year analyzed were included. ~~The same strategy was applied for the environmental data, with the exception of the carbonate system parameters. Planktic foraminifera calcification variability across the twelve-year record varied depending on the species (Fig. 5).~~

*G. bulloides* MBW$_{area}$ showed a slight ~~constant~~ decrease from 1994 to 2000 and a slight increase from 2000 to 2006. Over the studied interval, the lowest value is reached in the year 2000 and the highest in 2004. Lowest mean annual values were reached during years 2000 and 2005 (3.9 and 3.85 µg, respectively).

On the other hand, *N. incompta* MBW$_{area}$ showed ~~little to none interannual variability,~~ a slight calcification reduction with the highest variability in recent years. Both maximum and minimum values are displayed in recent years: 2004 and 2005 respectively. Mean yearly MBW$_{area}$ values reach a maximum in 2003 (4.4 µg) and a minimum in 2005 (3.2 µg).

Finally, *G. truncatulinoides* MBW$_{area}$ showed a different pattern, with an overall steep calcification increase throughout the record. Minimum calcification values are observed in 1996, which is also the year with the lowest mean MBW$_{area}$ (16.5 µg) observed. Maximum value is displayed in 2003, and its mean value is also the highest of the record (26.1 µg).

All environmental parameters showed variations across the years. Sea Surface Temperatures (SSTs) displayed a slight but constant decrease over the years~~, from 13.8 to 13.4 °C~~, while salinity showed a slight increase, mainly since 2002. From late 2000 until late 2002, phosphate and nitrate concentrations were exceptionally low (Fig. 5). This feature has already been described in the Gulf of Lions (Meier et al., 2014). ~~However, data from the carbonate system showed variations across the years.~~ Between the 2 periods for which direct *in situ* carbonate system parameters measurements were available, 1998 to 2000 and 2003 to 2005 (Fig. 5), CO$_3^{2-}$ dropped by 10-15 µmol/kg, DIC increased by 40 to 60 µmol/kg, leading to a pH decrease of 0.02 to 0.025.

**4.4. Sediment trap, core tops and sediment cores MBW patterns**

Foraminifera weights analyzed in core tops and sediment cores from the NW part of the Mediterranean (Fig. 6) and radiocarbon dating allowed a further insight on ~~reduced~~ foraminifera calcification during the Holocene.

Flux-weighted MBWs (see section 3.4) from Planier sediment trap for the three planktic species were 4.1 µg for *G. bulloides*, 3.3 µg for *N. incompta* and 22.3 µg for *G. truncatulinoides* (Fig. 6).

Data from Planier core-top showed higher mean MBW$_{area}$ values: 5.3 µg, 4.65 µg and 35.4 µg. ~~$^{14}$C dating in this core-top revealed a date of 489 years (± 59 years). Overall, in the last 489 years~~. $^{14}$C dating carried out in this core-top was out of the

calibration range (see section 3.7 for more details), implying that this sample could be considered post-industrial. Compared to the flux-weighted MBWs from the sediment trap, *G. bulloides* weight has been reduced by 1.2 μg, *N. incompta* by 1.3 μg and *G. truncatulinoides* by 12-13 μg.

Located west of Planier site, Lacaze Duthiers sediment core mean MBWs were: 4.99 μg for *G. bulloides*, 4.14 μg for *N. incompta*, and 32.9 μg for *G. truncatulinoides*. ~~$^{14}$C dating was carried out on the core-top sample of this sediment core that displayed a date of 460 years (± 59 years).~~ $^{14}$C analysis displayed a post-industrial age (see section 3.7) for this sample and corresponding MBWs from this sample for *G. bulloides*, *N. incompta* and *G. truncatulinoides* respectively were: 4.7 μg, 4.2 μg and 34 μg. Overall, compared to the data from the sediment trap, this corresponds to a 0.6 μg weight loss for *G. bulloides*, 0.9 μg for *N. incompta* and 12.2 μg for *G. truncatulinoides* in ~~460 years.~~

Finally, in the Gulf of Minorca, northwest of Planier site, Minorca sediment core mean MBWs were: 5.4 μg for *G. bulloides*, 4.5 μg for *N. incompta* and 36.3 μg for *G. truncatulinoides* (Fig. 6). $^{14}$C dating on this core top was carried out on an intermediate depth (see section 3.7) due to the lack of availability of enough specimens in the core-top and displayed a date of 1560 calendar years BP (Table 1). Corresponding MBWs for this sample were 5.4 μg, 4.9 μg, 38.2 μg for the three species. Therefore, the weight reduction compared to the sediment trap flux-weighted MBWs are: 1.3 μg for *G. bulloides*, 1.6 μg for *N. incompta* and finally, 16 μg for *G. truncatulinoides*.

**Table 4.** Mann-Whitney variance test results between the MBW$_{area}$ of the different sites for the three species. Significant values (p<0.05) are set in bold.

| | | PLA ST | PLA CT | LCD SC | MR 3.1.A |
|---|---|---|---|---|---|
| | | | | MBW$_{area}$ | |
| *G. bulloides* | | | | | |
| PLA ST | | | 0.110 | **0.003** | **7.86e$^{-13}$** |
| PLA CT | MBW$_{area}$ | 0.110 | | 1 | 1 |
| LCD SC | | **0.003** | 1 | | 0.114 |
| MIN SC | | **7.86e$^{-14}$** | 1 | 0.114 | |
| *N. incompta* | | | | | |
| PLA ST | | | 0.438 | 0.890 | **2.59e$^{-5}$** |
| PLA CT | MBW$_{area}$ | 0.438 | | 0.342 | 1 |
| LCD SC | | 0.890 | 0.342 | | **0.034** |
| MIN SC | | **2.59e$^{-5}$** | 1 | **0.03** | |
| *G. truncatulinoides* | | | | | |

| | | PLA ST | PLA CT | LCD SC | MIN SC |
|---|---|---|---|---|---|
| **PLA ST** | | | 0.120 | **0.003** | **3.13e-15** |
| **PLA CT** | MBW$_{area}$ | 0.120 | | 0.644 | 1 |
| **LCD SC** | | **0.003** | 0.644 | | **0.01316** |
| **MIN SC** | | **3.13e-15** | 1 | **0.013** | |

699

A Mann-Whitney variance test (see section 3.6) was carried out in order to analyze the variance between the different MBW$_{area}$ datasets (Table 4) from the different sites. MBW$_{area}$ data from the sediment trap appeared to have a significantly different variance compared to the MBW$_{area}$ from Menorca sediment core for the three species ($3.13e^{-15} < p < 2.59e^{-5}$), however, differences between the sediment trap data and the with Lacaze-Duthiers sediment core were only significant for *G. bulloides* and *G. truncatulinoides* (p=0.003). Concerning differences between the Planier sediment trap and the underlying core-top, no significant differences were observed for any of the species ($0.11 < p < 0.438$), most likely due to the small number of samples from the latter site: only 2 samples were available. Note that the differences between the sediment cores MBW$_{area}$ datasets differ according to the site and species. In the case of *G. bulloides*, no significant differences are observed between Planier core-top, Lacaze-Duthiers sediment core and Menorca sediment core. In the case of *N. incompta* and *G. truncatulinoides*, differences between Lacaze-Duthiers and Menorca sediment core are significant ($0.013 < p < 0.03$), although on lower orders of magnitude compared to the differences between the sediment trap and sediment cores datasets (Table 4). This demonstrates that the difference between the sediment trap MBW$_{area}$ dataset and the seabed sediments MBW$_{area}$ datasets is greater than the difference between the different seabed MBW$_{area}$ datasets.

## 5. Discussion

**5.1. Seasonal controls on planktic foraminifera shell calcification in the NW Mediterranean**

As described in section 4.2, the seasonal variability of MBW$_{area}$ displays important differences across the three species analyzed. The different seasonal pattern in MBW$_{area}$ is reflected by the lack of correlation between the seasonal pattern of MBW$_{area}$ of the different species, i.e., r= -0.23 (p>0.05) between *G. bulloides* and *N. incompta* and r= 0.16 (p>0.05) between *G. bulloides* and *G. truncatulinoides*. Only the seasonality of *N. incompta* MBW$_{area}$ and *G. truncatulinoides* MBW$_{area}$ share some similarities, as reflected in the significant and positive correlation (r= 0.66; p<0.05). In order to examine the main controls on foraminifera seasonal calcification in the Gulf of Lions, here we compare the seasonal variability of planktic foraminifera

calcification with foraminifera fluxes previously estimated for the Planier sediment trap (Rigual-Hernández et al., 2012) satellite data for the studied site and a suite of environmental parameters measured at the DYFAMED site (see section 3.4). Furthermore, GAM have been generated for all three species (see Supplementary figs. 3,4 and 5) and the environmental parameters considered here in order to give a further insight on the potential factors controlling the MBW$_{area}$. These models showed that *G. bulloides* and *G. truncatulinoides* seasonal calcification trends are significant (p=0.05 and p=2.4e-5 respectively). On the other hand, *N. incompta* seasonal trend does not appear to be significant (p=0.14).

**Table 5**. Correlation matrix of seasonal (monthly) test weights and the environmental parameters from Planier (sediment trap and satellite data) and DYFAMED site (see section 3.4). Significant correlations (p<0.05) are set in bold.

| Parameters | | Planier site data | | | | | | | | DYFAMED site data | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | G. bull. | N. inc. | G. truncat. | G. bull. | N. inc. | G. truncat. | Chl-a | SST | Salinity | [NO$_3$] | [PO$_4$] | pH | [CO$_3$] | [CO$_2$] |
| | | MBW$_{area}$ | | | Fluxes | | | | | | | | | | |
| G. bull. | MBW$_{area}$ | 1 | 0.232 | 0.167 | 0.012 | 0.027 | 0.152 | 0.318 | -0.32 | -0.163 | 0.292 | 0.33 | 0.096 | 0.189 | 0.243 |
| N. inc. | | -0.232 | 1 | **0.667** | **-0.582** | -0.407 | -0.405 | -0.484 | **0.688** | 0.368 | 0.272 | 0.235 | -0.35 | 0.474 | -0.28 |
| G. truncat. | | 0.167 | **0.667** | 1 | **-0.905** | **-0.725** | **-0.666** | **-0.585** | **0.672** | -0.299 | 0.258 | 0.512 | 0.113 | **0.732** | 0.541 |

Here, we first approach seasonal shell calcification by considering the Optimum Growth Conditions (OGC). Previous studied have defined these conditions on a wide variety of ways: abundance of foraminifera, the chlorophyll-a concentration and even nutrients concentration (de Villiers, 2004; Schiebel et al., 2001; Schiebel and Hemleben, 2017). Therefore, we aim to explore the impact of these parameters as OGC on the shell calcification.

Among all the environmental parameters, de Villiers; (2004) suggested that shell calcification, and therefore MBWs, is primarily controlled by the OGC that can be defined as the most suitable environmental conditions for the development of a given planktic foraminifera species. Based on the latter study, it could be expected that favorable environmental conditions for foraminifera growth would lead to both greater shell fluxes and enhanced shell calcification. Our correlation analysis shows different relationships between foraminifera abundance (as inferred from foraminifera shell fluxes) and shell weight. In particular, *G. bulloides* MBW$_{area}$ shows no correlation with its fluxes (Table 5), while both *N. incompta* and *G. truncatulinoides* exhibit a negative correlation between MBW$_{area}$ and their species fluxes although only significant for the latter species (r= -0.4, p>0.05 and -0.66,

764  p<0.05 respectively). GAM results (see Supplementary figures 3, 4 and 5) support
765  these observations, with shell flux showing a stronger effect on the calcification for
766  *G. truncatulinoides* compared to the other two species fluxes.
767  According to the OGC theory, species calcification patterns vary according to the
768  species and their fluxes. Interestingly, *G. truncatulinoides* calcification correlates
769  negatively and significantly with all three species fluxes, a pattern opposite to what
770  the OGC theory predicts (de Villiers, 2004), i.e., optimum ecological niche is
771  associated with enhanced calcification. Thus, a possible explanation reconciling our
772  observations with the OGC theory may be that *G. truncatulinoides* tend to prioritize
773  energy allocation toward growth and reproduction at the price of a reduced
774  calcification. As described previously (see section 3.3), this species life cycle is
775  complex as it migrates through different depth levels of the water column throughout
776  the year. It is thought to reproduce once a year in winter in subtropical waters and it
777  has been speculated that nutrient availability and the lack of predation could explain
778  this strategy. Therefore, the observation of peak calcification in autumn/winter (Figs.
779  3 and 4) may reflect the migration of adults - that have spent time in deeper waters
780  developing a thick calcite crust - to shallower waters in late autumn to winter to
781  undergo reproduction. During this interval, the other major species display low
782  abundances in the water column, which could allow *G. truncatulinoides* to reproduce
783  due to the lack of competition. *N. incompta* calcification displays a similar pattern, a
784  negative correlation with all three species, but with a lower level of significance. It's
785  MBW$_{area}$ correlates negatively and significantly ($p<0.05$) with *G. bulloides* flux, but
786  its fluxes correlate positively and significantly with the latter species fluxes (see
787  Supplementary table 2). This is interesting, as it may highlight interspecific relations.
788  First, this could lead to the assumption that when *G. bulloides* dominates the
789  assemblages, *N. incompta* also displays a high abundance (Rigual-Hernández et
790  al., 2012). Then, it could show that when conditions are favorable, *N. incompta*
791  reproduces at a higher rate at the price of thinner shells (Table 1). This agrees with
792  *N. incompta* life cycle, which is known to be outnumbered by opportunistic species
793  when nutrients supply is high (Schiebel et al., 2002), but dominate the assemblages
794  when stratified waters are set,  therefore when conditions are favorable or when in
795  cohabitation with opportunistic species, it could focus on its reproduction. Note that
796  *G. truncatulinoides* and *N. incompta* MBW$_{area}$ correlate positively and significantly
797  ($p<0.05$), showing a similar calcification pattern on a seasonal scale.
798  An alternative proxy for OGC that may be considered is chlorophyll-*a* concentration.
799  Chlorophyll is considered an indicator of the algal biomass concentration, which is
800  known to represent a large part of some foraminifera species diet, specially for *G.*
801  *bulloides* (Schiebel and Hemleben, 2017). Our data shows a positive but not
802  significant correlation between chlorophyll concentration and *G. bulloides* MBW (r=

803    0.32, p>0.05), suggesting a negligible influence of phytoplankton concentration on
804    *G. bulloides* calcification. A stronger trend would be expected under the OGC theory
805    as algae are a vital part of its diet (Hemleben et al., 1989). This lack of correlation
806    between *G. bulloides* and chlorophyll-*a* has already been described (Weinkauf et al.,
807    2016). We speculate that *G. bulloides* may preferentially feed on certain groups of
808    phytoplankton which changes in seasonal abundance in the photic zone do not
809    necessarily follow the seasonal pattern of total chlorophyll concentration (Marty et
810    al., 2002). In the case of *N. incompta* and *G. truncatulinoides*, correlations between
811    $MBW_{area}$ and chlorophyll-*a* are negative and stronger although only significant for the
812    latter species (r= -0.58, p<0.05). GAM results further support these observations
813    (see Supplementary fig. 5), with chlorophyll-a showing a significant impact on *G.*
814    *truncatulinoides* calcification. This observation indicates that optimum calcification
815    conditions for *G. truncatulinoides* are reached at times of minimum annual algal
816    biomass concentration in the photic zone. It is possible that, due to its deeper habitat
817    (Schiebel and Hemleben, 2017), *G. truncatulinoides* feeds on phytoplankton
818    dwelling in subsurface levels of the water column. In fact, a deep chlorophyll
819    maximum is known to develop during large part of the year in the Northwestern
820    Mediterranean (Estrada et al., 1993) but its presence is not detected by satellites.
821    This interpretation is in agreement with earlier work by Pujol and Vergnaud Grazzini
822    (1995) who found peak abundances of this species during the summer below the
823    thermocline.

824
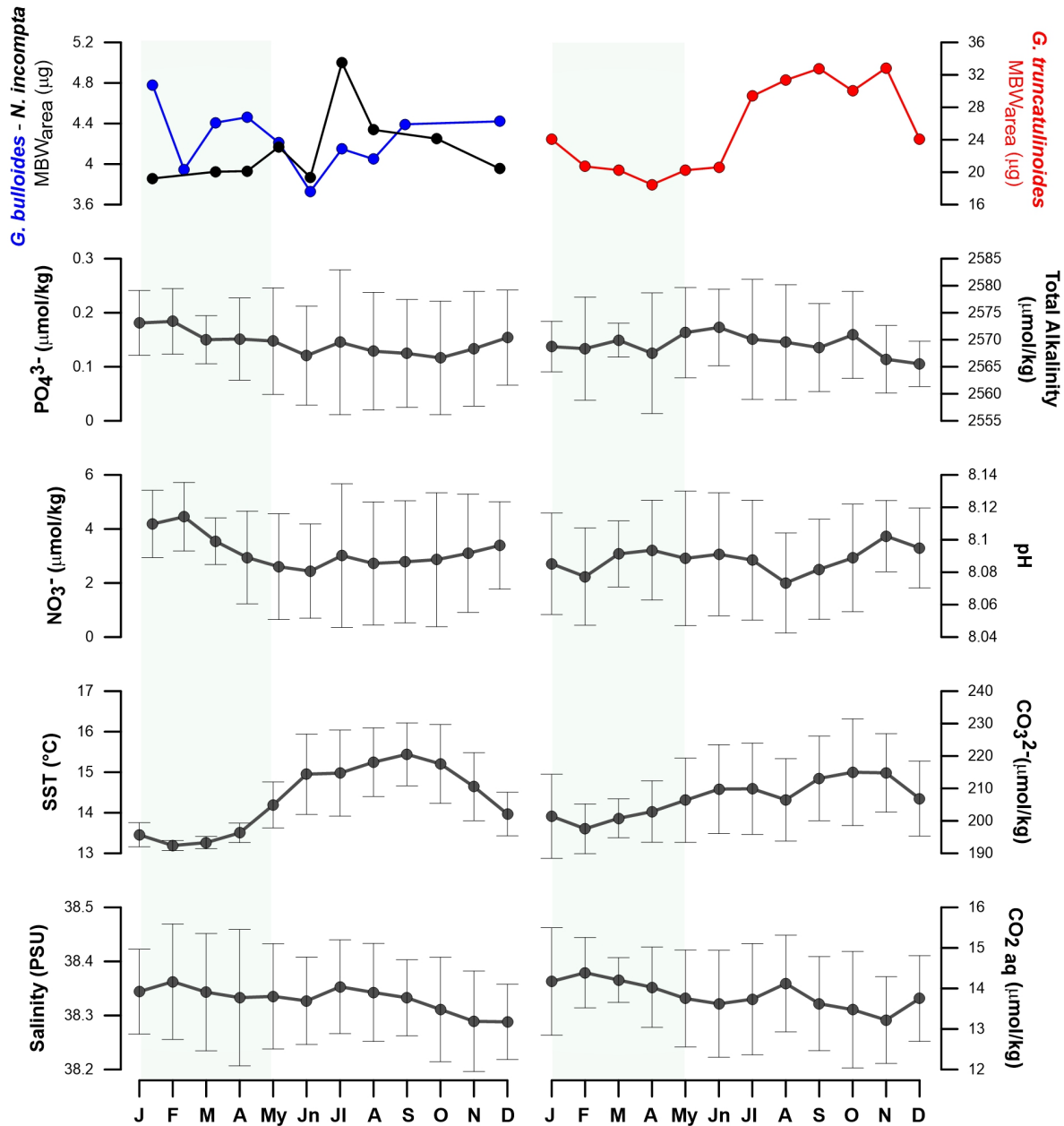
**Figure 4.** *G. bulloides, N. incompta* and *G. truncatulinoides* seasonal mean
monthly MBW$_{area}$ variations compared with Planier environmental data and
the resampled seasonal signal of environmental parameters from the
DYFAMED site across a composite year.

Something to consider when using chlorophyll as an OGC proxy is that it can be
confounded with the nutrient concentrations. Previous studies have described that,
in those settings where foraminifera abundance covaries with nutrient
concentrations, then nutrients are probably a better OGC proxy than chlorophyll
concentrations (Schiebel et al., 2001). In turn, the correlation of nutrients (nitrates
and phosphates) with fluxes were positive for all three species, although only

835 significative (p<0.05) for *G. truncatulinoides* abundance (r= 0.58 and 0.59 for nitrates
836 and phosphates respectively). Although here we have first described the OGC as
837 species fluxes and then as the chlorophyll-*a* concentration, it is important to
838 remember that the niche and favorable conditions meant to be described by the OGC
839 for each species are multi-dimensional.
840 ~~It is important to~~ Note that nitrate and phosphate concentration variations were
841 closely linked to each other (r= 0.876, p<0.05) , making it difficult to determine if the
842 resulting effect on foraminifera calcification is due to the effect of a single driver or
843 to the combination of both. High phosphate concentrations are generally considered
844 an inhibitor for foraminifera calcification (Zeppenfeld, 2019). Evidences show that
845 calcite formation is inhibited by $PO_4^{3-}$ due to its adsorption on the calcite surface,
846 impeding its precipitation by blocking the crystal growth. Aldridge et al., (2012)
847 showed a negative effect of phosphate concentration on *G. bulloides* with $PO_4^{3-}$
848 concentrations of 0.1-0.4 $\mu$M. This feature has been shown in other calcareous
849 organisms such as coccolithophores (Paasche and Brubank, 1994) and calcifying
850 algae (Demes et al., 2009), resulting in a higher growth and calcification under
851 controlled $PO_4^{3-}$. On the other hand, no studies show a reduced calcification in
852 marine organisms under high $NO_3^-$ values (Fig. 4). Our work shows that nutrient
853 concentrations (both nitrates and phosphates) do not correlate significantly with any
854 of the three species MBW studied, and this observation is supported by the GAM
855 results which do not show any significant effect of nutrients concentrations on the
856 calcification. $NO_3^-$ correlations were not significant for all three species, while $PO_4^{3-}$
857 correlations were also very weak, with the exception of the one with *G.*
858 *truncatulinoides* MBW$_{area}$ (r= -0.512, p= 0.07) (Table 5). ~~Although the p-value of this~~
859 ~~correlation is slightly higher than what we use as threshold for significance (i.e.,~~
860 ~~0.05), the negative correlation between *G. truncatulinoides* MBW$_{area}$ and phosphate~~
861 ~~could be considered almost significant. Thus, our data suggest that enhanced [PO$_4^{3-}$~~
862 ~~] levels could act as an inhibitor for *G. truncatulinoides* calcification in our study~~
863 ~~region.~~
864 Previous studies have suggested that salinity may have an influence on foraminifera
865 calcification. Zarkogiannis et al., (2022) found that salinity may control calcification
866 of certain foraminifera species in the central Atlantic region. However, our data
867 suggest that the role of salinity on calcification in our study region is unlikely since
868 its seasonal amplitude is tiny (0.1 PSU; Fig. 4). This idea is supported by the lack of
869 correlation between salinity and MBW$_{area}$ for the three species studied (Table 5) and
870 the GAM results, in which salinity was the parameter that displayed the lowest effect
871 on calcification.
872 Temperature (Sea Surface Temperature) has been described as a major factor that
873 controls the size (Schmidt et al., 2004) and porosity (Burke et al., 2018) of planktic

874  foraminifera, therefore it could represent a major control factor on shell calcification
875  in the NW Mediterranean. In particular, calcification could be positively linked to
876  temperature through different mechanisms: (i) warmer temperatures have been
877  shown to increase enzymatic activity and therefore enhanced growth and
878  calcification rates (Spero et al., 1991); (ii) Lombard et al., (2011) stated that higher
879  temperatures could also increase feeding and ingestion rates, but it remains unclear
880  if this could result in a calcification rate increase. Our data revealed that SST
881  correlates negatively, but without significance with *G. bulloides* calcification, while
882  correlations between SST and *N. incompta* and *G. truncatulinoides* were both
883  positive and significant (r= 0.69 and 0.67 respectively, p<0.05). GAM results also
884  displayed a positive and the most significative effect of the SST on these two
885  species. These findings highlight that SSTs are one of the main factors affecting *N.*
886  *incompta* and *G. truncatulinoides* calcification among the parameters considered
887  here. These trends could be expected considering the relationship between
888  temperature and nutrient concentrations (r= -0.69 and -0.70 for $NO_3^-$ and $PO_4^{3-}$
889  respectively) and the correlations between the three species $MBW_{area}$ and nutrients
890  concentrations (Figure 5). Finally, in addition to having an impact on the size and
891  calcification of the planktic foraminifera, temperature is well known as a major control
892  of the carbonate system, due to the increased solubility of atmospheric $CO_2$ at lower
893  temperatures, and therefore it could have an indirect effect on foraminifera
894  calcification by affecting the carbonate system.
895  Data for the carbonate system were only available for years 1998 to 2000 and 2003
896  to 2005 and, therefore gaps comprised in these years were filled with estimates
897  using the CO2sys macro (see section 3.6 for more details). However, note that the
898  data available for these parameters was relatively smaller compared to the other
899  parameters and may have prevented detection of other significant relationships. The
900  relationship between $CO_3^{2-}$ and MBW has been described in previous studies
901  (Barker and Elderfield, 2002; Marshall et al., 2013) and the bulk of evidence indicates
902  that foraminifera MBWs to be positively linked with $CO_3^{2-}$ concentrations (Aldridge et
903  al., 2012; Osborne et al., 2016). However, it appeared that planktic foraminifera
904  response to $CO_3^{2-}$ concentration was not uniform and varied across species (Beer
905  et al., 2010; Lombard et al., 2010). The trends between carbonate system
906  parameters and MBWs were similar to those observed when comparing MBWs with
907  temperature, highlighting the covariations between these two parameters (Fig. 4).
908  Our data showed that $CO_3^{2-}$ concentrations were not significantly correlated with *G.*
909  *bulloides* $MBW_{area}$ nor with *N. incompta*. Only *G. truncatulinoides* $MBW_{area}$ displayed
910  a clear significant correlation with $CO_3^{2-}$ concentration (r= 0.73, p<0.05), implying
911  that carbonate availability may represent a key control on *G. truncatulinoides* in the
912  Northwestern Mediterranean. On the other hand, $CO_2$ concentrations, excepting a

913  negative and almost significant relationship with *G. truncatulinoides* MBW$_{area}$ (r= -
914  0.54, p= 0.06), showed no correlation with the other 2 species MBWs. None of the
915  remaining carbonate system parameters (pH, alkalinity and calcite saturation),
916  exhibited a significant seasonal correlation with the MBWs. On the other hand, GAM
917  result (see Supplementary figs. 3, 4 and 5) did not show a significant impact of any
918  carbonate system parameters for any of the three species calcification. As stated
919  previously, the lack of data could have prevented the detection of further trends, but
920  considering the seasonal patterns of carbonate system parameters, a potential role
921  of the $CO_3^{2-}$ concentration could be expected.
922  In summary, seasonal correlations, trends and GAM showed that the environmental
923  parameters that displayed the highest correlation with MBW$_{area}$ vary according to the
924  species. *G. bulloides* calcification appeared to be affected mainly by interspecific
925  relations. *N. incompta* calcification showed to be mainly positively linked to the SST.
926  Finally, *G. truncatulinoides* calcification was positively linked with the SST and
927  potentially $CO_3^{2-}$ concentration, while OGC displayed a negative effect on its
928  MBW$_{area}$. OGC (considered as fluxes and chlorophyll-a concentration), SST and
929  potentially $CO_3^{2-}$ are the most likely parameters that control the seasonal changes
930  in calcification of the planktic foraminifera species studied here. The combined effect
931  of these parameters seems to control foraminifera calcification in the Gulf of Lions;
932  however, it should be considered that covariation between these parameters is
933  strong, and therefore it is difficult to isolate the effect of a single parameter.
934  Moreover, it is likely that the ecology and life cycle of the species could also be a
935  major factor affecting the response of the species calcification to the environmental
936  parameters variations. Our results are in agreement with earlier studies that stated
937  that OGC (de Villiers, 2004), temperatures and $CO_3^{2-}$ (de Villiers, 2004; Marshall et
938  al., 2013; Osborne et al., 2016) concentrations are the main factors that impact
939  calcification in planktic foraminifera, while the calcification response to those
940  parameters is species-specific, which is in agreement with the work of Weinkauf et
941  al, (2016).
942

### 5.2. Interannual trends in planktic foraminifera calcification

944  As stated previously, the Mediterranean Sea is a sensitive zone to atmospheric $CO_2$
945  accumulation (Ziveri, 2012) and is experiencing ongoing ocean acidification. On an
946  interannual time scale, different studies (Beer et al., 2010; Osborne et al., 2016)
947  have shown that sea surface warming and carbonate system parameters are the
948  most likely parameters to control calcification on key calcifying phytoplankton
949  species such as the coccolithophore *Emiliania huxleyi* organisms (Meier et al.,
950  2014). However, datasets from sediment traps that cover a wide span of years and
951  in which foraminifera weights have been analyzed are rare (Kiss et al., 2021),

952 therefore it is difficult to place our results in a more global context. Our GAM results
953 (see Supplementary figs. 3 and 4) showed that both *G. bulloides* and *N. incompta*
954 interannual patterns were non-significant. This is not surprising as the calcification
955 trends for these two species did not display a clear and marked variation over the
956 years, excepting a small mean calcification reduction (Fig. 3) and minimum
957 calcification values in 2004 and 2005 (Fig. 3 and Supplementary figs. 3 and 4). Our
958 analysis suggests that the trends observed in the carbonate system (Figure 4), i.e.,
959 the DIC rise and $[CO_3^{2-}]$ decrease may be responsible for the slight calcification
960 reduction in both *G. bulloides* and *N. incompta*, particularly in recent years. During
961 the second time span carbonate parameters were available (2003 to 2005), both of
962 these species started experiencing a calcification reduction (Figure 5). *G. bulloides*
963 displayed a relative maximum $MBW_{area}$ in 2004, corresponding to a relative
964 maximum in $[CO_3^{2-}]$ and relative minimum in DIC. While in 2005, calcification started
965 to decrease, according to the opposite pattern in $[CO_3^{2-}]$ and $[CO_2]$. *N. incompta*
966 started a decreasing calcification pattern from 2003 to 2005, matching the carbonate
967 system parameters trends in recent years. Our data showed that pH decreased from
968 a mean 8.12 for the 1998-2000 years to a mean 8.08 for the 2003-2005 years. This
969 pH decrease, combined with the carbonate system parameters seem to affect *G.*
970 *bulloides* and *N. incompta* calcification patterns in recent years, however, the degree
971 to which each species react to a change in these parameters differs.
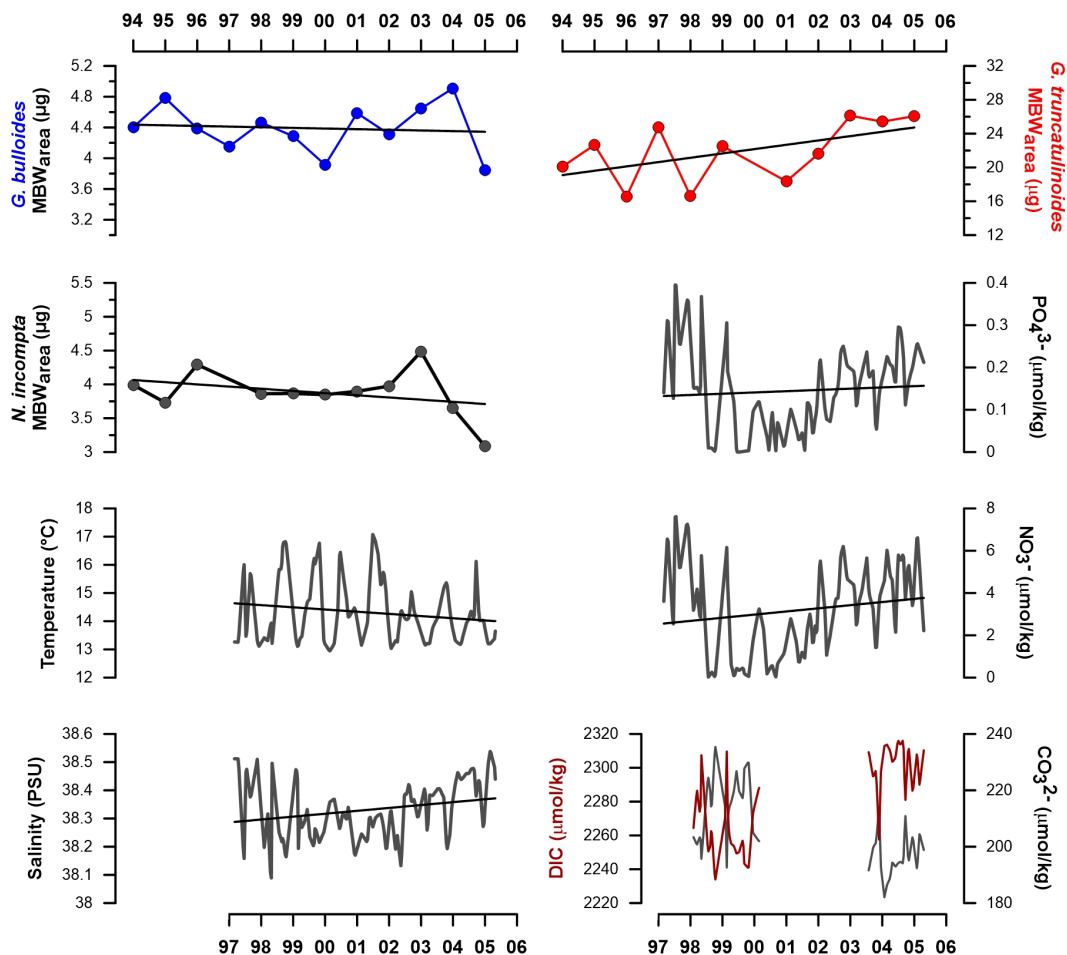
**Figure 5.** Interannual mean MBW$_{area}$ (*µ*g) values for *G. bulloides, N. incompta* and *G. truncatulinoides* from the high productivity period (see section 2) and Planier and DYFAMED environmental data variations across the record. Black lines represent the trends from the MBW$_{area}$ and resampled data. DIC represents "Dissolved Inorganic Carbon".

Notably, the trend in *G. truncatulinoides* is opposed to the previous two species and shows a steady and steep increase throughout our record. Over the analyzed time span, its MBW increased around 20% (equivalent to an increase of ~5 µg). According to the GAM results, the interannual calcification trend for this species is significant (see Supplementary figure 5). If this calcification increase continues on current trends, then the average MBW of *G. truncatulinoides* will double by 2024. Analysis of present *G. truncatulinoides* populations is urgently needed to assess if the observed trend held true during the last two decades. It is important to note that while *G. truncatulinoides* exhibits an intimate correlation with $CO_3^{2-}$ concentration on a seasonal scale, no clear correlation was found with the interannual changes of $CO_3^{2-}$ concentration. This feature is also supported by the GAM results. ~~In fact, our analysis suggests that the most likely environmental parameter exerting an influence~~

990   ~~on the calcification of this species is nitrate concentration (Figure 5).~~ A similar
991   enhancement in shell calcification has been described in the Balearic Sea for *G.*
992   *truncatulinoides* in high-resolution sediment cores (Pallacks et al., 2020), but also in
993   *Globorotalia inflata*. Taken together, our observations and the study mentioned
994   above, suggest that deep dwellers are unaffected by the recent ocean acidification
995   and changes in the carbonate system and that the recent change in one or several
996   environmental drivers may be stimulating the calcification of these species.
997   Here, we theorize that the interannual patterns presented in Figs. 3 and 5 mainly
998   reflect the seasonal changes in the regional oceanographic setting. As described
999   previously (see section 2 for more details), the Gulf of Lions is influenced by a strong
1000  seasonality. The recent SST decrease could be linked to an enhancement in water
1001  mixing, as cold and deep salty water reach up to the surface. This mechanism would
1002  be less intense during years 2000 to 2002, corresponding to a SST increase along
1003  with a salinity decrease and absolute minimums in nutrients concentrations (Fig. 5),
1004  as water stratificates, these are consumed by primary production. Finally, in recent
1005  years, water mixing seems to be reactivated, as SST keeps decreasing and nutrients
1006  concentrations increase again. This mechanism also affects the carbonate system
1007  parameters, as water mixing brings to surface deeper DIC enriched waters to the
1008  surface, coupled with a $[CO_3^{2-}]$ reduction. Our data shows that alkalinity patterns
1009  display similar tendencies to DIC, however, until the second time span covered by
1010  carbonate system data, alkalinity variations are proportionally higher than DIC
1011  variations (see Supplementary material), suggesting a water mixing phenomenon.
1012  On the other hand, DIC variations turn to be higher than alkalinity variations from
1013  2003 to 2005, suggesting an additional effect of carbon inputs on the carbonate
1014  system not reflected in the alkalinity data.
1015  Note that SSTs, despite showing a positive and significant correlation with *N.*
1016  *incompta* and *G. truncatulinoides* on a seasonal scale and the GAM showing a
1017  positive and significant effect on the calcification, did not follow the same pattern as
1018  the latter species. This observation implies that other mechanisms or parameters
1019  than the ones considered here may be affecting the MBW$_{area}$ on an interannual
1020  scale.
1021
1022  **5.3. Holocene core-top data comparison**
1023
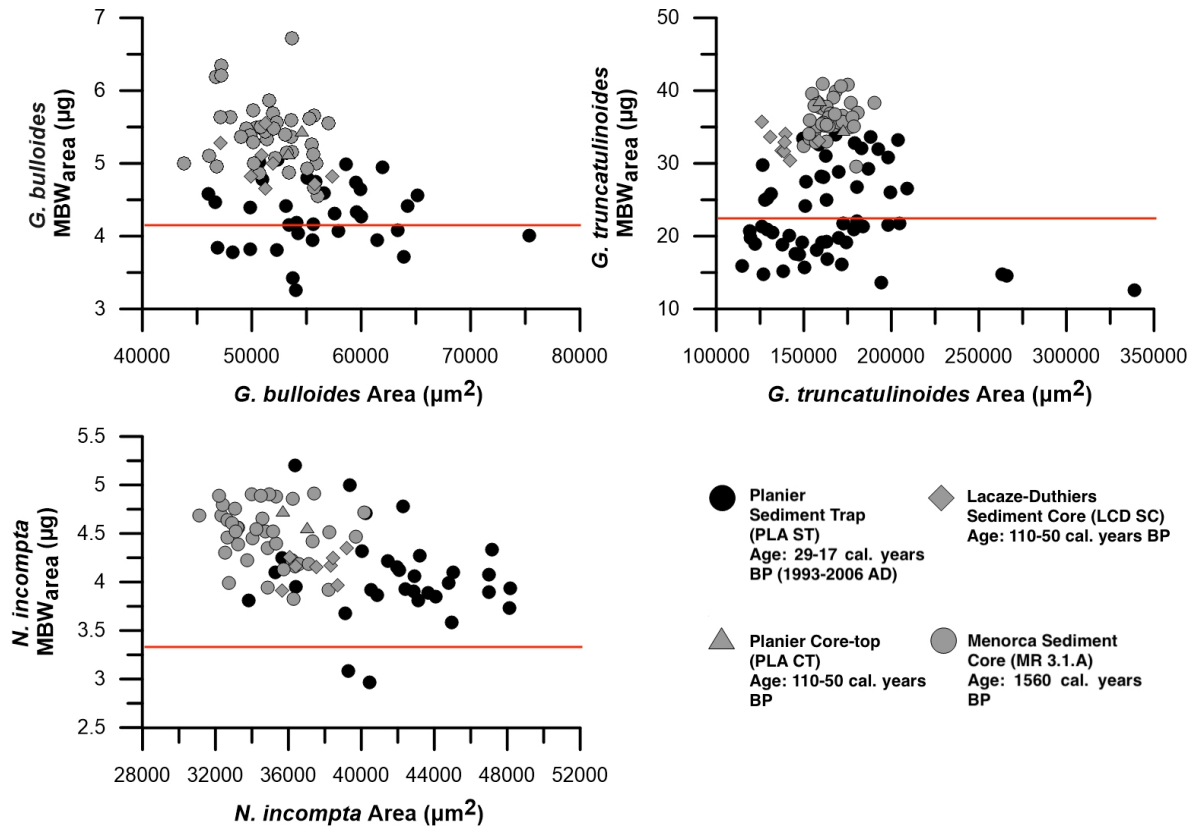
**Figure 6.** MBW<sub>area</sub> in μg and area in μm² comparison in the sediment trap (PLA ST), Planier core-top (PLA CT), and both Lacaze-Duthiers (LCD SC) and Minorca sediment core (MR 3.1.A). Black dots represent data from the sediment trap, while lighter colors represent data from the different seabed sediments. Red lines represent the flux-weighted values from the sediment trap.

The comparison of the well-preserved assemblages of planktic foraminifera in the pre-industrial and industrial Holocene-aged surface sediments with those collected by a long-sediment trap record offers a unique opportunity to assess the impact of recent environmental change on the calcification of calcareous zooplankton in the Mediterranean Sea (Fig. 6). However, when comparing data from sediment traps and seabed sediments, the possible role of calcite dissolution must be taken into account.

Calcite dissolution in the water column and/or on the sea floor could be invoked as a source of variability between the sediment trap and surface sediment data sets (e.g., Dittert et al., 1999). Therefore, in order to obtain meaningful interpretations from our data sets it is important to assess the possible role of dissolution in the preservation of planktic foraminifera shells. Several lines of evidence suggest, however, that calcite preservation does not represent an important source of bias in our study area. Firstly, the Mediterranean Sea is supersaturated with respect to calcite (Millero et al., 1979) and the location of all the analyzed samples is much

1046 shallower than the location of the calcite saturation horizon (Álvarez et al., 2014),
1047 therefore, calcite dissolution seems unlikely (Schneider et al., 2007). Secondly,
1048 several sediment trap studies have documented that calcareous plankton
1049 experience negligible dissolution in their transit from the surface ocean to the sea
1050 floor (Beaufort et al., 2007; Moy et al., 2009; Rigual-Hernández et al., 2020). Thirdly,
1051 SEM and microscopic observations of all 3 species in samples from both the
1052 sediment traps and sediment cores showed no sign of dissolution and foraminifera
1053 were well preserved (see Supplementary fig. 6). These arguments suggest that
1054 calcite dissolution does not represent an important control in the weight of the
1055 planktic foraminifer shells in the analyzed samples. However, it has been
1056 documented that when dissolution takes place, the thinnest shells are affected first
1057 (Berger, 1970) while the heaviest and more calcified specimens remain. In our study,
1058 the specimens from the sediment trap were lighter that the ones from the sediment
1059 cores, therefore, this is important to acknowledge as dissolution cannot be
1060 completely ruled out as a possible source of variability between the surface sediment
1061 and sediment trap data sets.
1062 Overall, the lower shell weights of the foraminifera collected by the traps strongly
1063 suggest that the three planktic foraminifera species have experienced a reduction in
1064 their calcification since the industrial era and/or late to recent Holocene. While the
1065 shell weight of each species measured in the sediments show some variability
1066 across seabed sediments (Fig. 6), our data suggest an overall reduction of 18-24%
1067 for *G. bulloides*, 20-27% for *N. incompta*, and 32-40% for *G. truncatulinoides.* It is
1068 important to note that the range of shell weight variability across core-tops and
1069 sediment cores (4.5-6.7 $\mu$g and 0.37 $\mu$g typical deviation for *G. bulloides*, 3.8-4.9 $\mu$g
1070 and 0.23 $\mu$g typical deviation for *N.incompta*, and 29.5-40.9 $\mu$g and 2.6 $\mu$g typical
1071 deviation for *G. truncatulinoides*) is substantially lower than the difference with the
1072 sediment trap data (3-5 $\mu$g and 0.5 $\mu$g typical deviation for *G. bulloides*, 2.9-5.2 $\mu$g
1073 and 0.5 $\mu$g typical deviation for *N. incompta* and 12-35 $\mu$g and 6 $\mu$g typical deviation
1074 for *G. truncatulinoides*), implying that the shell weight of recent foraminifera
1075 populations for the three species is lower than anywhere in the NW Mediterranean
1076 in the pre-industrial and post-industrial times. The source of the variability across
1077 core tops and sediment cores is most likely caused by the different age of the
1078 samples, ranging from 1560 cal. years BP at Minorca mid-depth (Table 1) sample to
1079 post-industrial at Planier and Lacaze-Duthiers core-tops, and the different
1080 environments associated to the location of each core top.
1081 A non-parametric two-way Mann-Whitney test (see sections 3.6 and 4.3) showed
1082 that the sediment trap MBW$_{area}$ dataset was significantly different (p<0.05) from MR
1083 3.1.A and non-different from PLA CT for all three species studied here (Table 5).

1084 Something to consider when comparing recent sediment trap data with pre-industrial
1085 Holocene data is the life cycle of the species. As all the species analyzed presented
1086 some reduction in shell calcification, the degree to which the different specimens
1087 responded varied. The greatest weight reductions were observed for *G.*
1088 *truncatulinoides* populations, while *G. bulloides* populations exhibited the lowest
1089 weight loss.

1090 Previous work stated that those species hosting photosynthetic algal symbionts
1091 exhibit a higher tolerance to environmental changes that may affect their calcification
1092 (Lombard et al., 2009). This is due to the fact that these symbionts can modify the
1093 sea water chemistry that is close range to the shell, allowing a calcification
1094 enhancement. Of the species studied here, none are known to be symbiont bearing
1095 species, with the possible exception of *G. bulloides*, therefore, they are among the
1096 most vulnerable foraminifera species to any sea water chemistry change.

1097 It has been described that some morphotypes of *G. bulloides* do actually host
1098 bacterial endobionts in their cytoplasm (Bird et al., 2017). The later work showed
1099 that high amounts of *Synechococcus*, a cyanobacteria, were found in morphotype
1100 IId specimens *of G. bulloides* from the California coast. Although no such
1101 observations have been reported on morphotype Ib, the dominant *G. bulloides*
1102 morphotype in the Mediterranean sea (Schiebel and Hemleben, 2017), this could be
1103 relevant as bacterial photosynthetic activity would interact on the close range
1104 seawater chemistry by removing $^{12}CO_2$ and therefore impacting the $^{13}C/^{12}C$ ratios in
1105 the surrounding dissolved $CO_2$. Moy et al., (2009) work in the Southern Ocean,
1106 showed a 30-35% calcification reduction for *G. bulloides* during the industrial era.
1107 Our study shows that such a similar reduction in *G. bulloides* MBW$_{area}$ (i.e., a mean
1108 20% taking into account the 3 sites studied) has also taken placed in the
1109 Mediterranean Sea.

1110 Even though the species studied were different in Fox et al., (2020), and that shell
1111 thickness was analyzed, that work showed a massive shell reduction for *N. dutertrei*
1112 (around 75%) and a smaller reduction for *G. ruber* (around 20%). *N. incompta* weight
1113 reduction in this study is around 25%, despite that life cycles are different between
1114 these species, our results come in the same line.

1115 Data for *G. truncatulinoides* calcification comparison between pre-industrial
1116 Holocene and post-industrial Holocene is scarce. One of the few available studies is
1117 the one of Pallacks et al., (2020) in the western Mediterranean sea using pre-
1118 industrial data and recent foraminifera weight data obtained from high resolution
1119 core-tops. Size-normalized weights showed that all the species calcification
1120 decreased since the onset of the industrial revolution, an observation that is
1121 supported by our data (Fig. 6). *G. truncatulinoides* showed a 24% weight reduction,
1122 which is a lower reduction than what is shown in our study (around 35% MBW$_{area}$

decrease), but shows a similar trend. Taken together, all these observations suggest that a decrease in major planktic foraminifera calcification is not only a regional feature but a global scale process.

On a more regional scale, Hassoun et al., (2015) documented the ongoing changes in seawater carbonate speciation in the Mediterranean waters. In the latter work, the distributions of anthropogenic $CO_2$ showed that all Mediterranean water masses have already experienced ocean acidification. This effect was more pronounced in the intermediate to deep masses (300-500m and >500m respectively) in the western basin, which translated into a minimum pH reduction of 0.1 in this part of the Mediterranean. As stated previously, over the years in which carbonate parameters were retrieved from the DYFAMED database, pH was reduced, DIC showed a marked increase and $[CO_3^{2-}]$ displayed a decrease. Taken together these observations and our data, it is possible that the observed changes in foraminfera calcification could have been totally or partially driven by the ongoing ocean acidification in the Mediterranean.

Moreover, the largest calcification reduction is observed between the seabed sediments and the sediment trap, this means that the highest calcification reduction has taken place between post-industrial Holocene and recent Holocene (i.e. the reduction between LCD SC/PLA CT and PLA ST) (Fig. 6). This could be explained with the "Great Acceleration theory". The Great Acceleration is a term used to describe the trends in $CO_2$ emissions and the associated temperature changes as consequences of the human impacts on the atmosphere since the 1950s (Head et al., 2022a, 2022b).

However, other important changes in physical and chemical parameters co-occur with ocean acidification, and therefore should be also considered. Based on the seasonal and interannual patterns of the SST in the Gulf of Lions (Figs. 4 and 5), temperature trends could also be invoked as a likely parameter to affect calcification here. As shown by the correlations (Table 5) and the GAM results, SSTs are one of the most likely parameters to affect calcification on different timescales. However, on a pre-industrial to post-industrial timescale, the effect of the SST on the foraminifera calcification may be hard to evaluate due to the effect of the latter on the carbonate system parameters such as $CO_2$ and $CO_3^{2-}$ concentration in the water But note that the Mediterranean is considered to be warming at a faster rate than the global average (Hassoun et al., 2015; Lazzari et al., 2014). Calcification data from the sediment trap has been flux-weighted (see section 3.4) in order to be compared with the sedimentary calcification data, therefore, this data could be affected by a change in the incoming foraminifera flux (de Moel et al., 2009). In this line, the Gulf of Lions, presents a marked seasonality (see section 2) and the both the mass fluxes (Heussner et al., 2006) and foraminifera fluxes present strong

seasonal variations. Parameters such as the North Atlantic Oscillation index, the river runoff and the intensity of the seasonal water cascading process have been suggested to play a role on planktic foraminifera production and export (Rigual-Hernández et al., 2012). The later study shows that most of the species flux showed a yearly uni-modal distribution, but the flux values and distribution remained fairly constant over the years. This highlights that, in our study zone, a major change in the foraminifera flux affecting the flux-weighted calcification value is unlikely.

## 5.4. Influence of environmental variability on MBW$_{area}$ across different time scales

Our results show that the influence of environmental parameters over the different time scales studied is not constant and depends on the species, the environmental driver and timescale.

In the case of *G. bulloides*, our data suggest that OGC and inter-specific relationships seem to affect its MBW on a seasonal scale, carbonate system seems to play a major role while on an interannual and on a pre/post-industrial time scales. *N. incompta* calcification seems affected by OGC, inter-specific relationships and SST on a seasonal scale, while on longer time-scales carbonate system appears to play preponderant role. Finally, *G. truncatulinoides* calcification seems positively correlated to carbonate system and SSTs and negatively with the OGC on a seasonal scale. However, these patterns seem to have an opposite effect on an interannual scale, as *G. truncatulinoides* calcification shows a clear increase while carbonate system parameters become less and less favorable for calcification. In turn, on a pre/post-industrial Holocene time scale, its MBW$_{area}$ seem to be affected by regional processes such as OA and warming.

Interestingly, this may suggest that other parameters that the ones considered in this work could also influence planktic foraminifera calcification on different time scales. Factors such as changes in the regional oceanographic processes (Cisneros et al., 2019; Durrieu de Madron et al., 2017) affect the physical and chemical properties of the water column and hence, could impact the life cycle of the species studied here. Also, while *G. bulloides* can either present regular or encrusted forms; *N. incompta* and *G. truncatulinoides* are crust forming species. In our study, *G. bulloides* individuals are mainly regular forms, but encrusted individuals were identified in both the sediment trap and seabed sediments. It is out of the scope of this work to focus on the effect of the crust on the species MBW, however, Osborne et al., (2016) study showed that encrusted *G. bulloides* individuals are around 20-30% heavier than the regular ones. Finally, reproductive strategy could be invoked as a factor affecting calcification. Planktic foraminifera mainly reproduce by gametogenesis, however, asexual reproduction has been documented to happen on a rare but constant rate

1201 (Meilland et al., 2021; Takagi et al., 2020). It has been theorized with overwintering
1202 the polar species *N. pachyderma* that if all of the individuals reproduce with an
1203 asexual strategy, they would double their size populations in 2 generations, with
1204 observations reporting the growth of extra chambers (Meilland et al., 2022).
1205 However, it remains to be shown if this phenomenon only affects polar species in
1206 such proportions and if other species show similar patterns.

## 6. Conclusions

The variability in shell calcification of three planktic foraminifera species (*G. bulloides*, *N. incompta* and *G. truncatulinoides*) was studied in the northwestern Mediterranean Sea at different time scales using sediment trap and seabed samples. The analysis of 273 samples and more than 4000 individuals revealed that:

i. The Sieve Based Weight (SBW) method is not a reliable tool as calcification indicator due to the influence of morphometric parameters on foraminifera weight. The Measured Based Weight (MBW) technique, on the other hand, shows little to negligible influence of the morphometric parameters, and therefore, can be considered a reliable calcification proxy.

ii. Analysis of the seasonal variability of planktic foraminifera calcification revealed important differences between species. *G. bulloides* exhibited peak calcification during winter, *N. incompta* during mid-summer and *G. truncatulinoides* during late summer to autumn.

iii. Interannual analysis suggest that *G. bulloides* and *N. incompta* did not display any significant pattern between 1994 and 2005, on the other hand, *G. truncatulinoides* displays a constant and steady calcification increase over recent years.

iv. Sediment trap and seabed sediment data comparisons between pre-industrial, post-industrial and recent Holocene assemblages shows that all three species experienced a clear and conspicuous calcification reduction with modern *G. bulloides* populations being 18-24% less calcified and a reduction of 20-27% and 32-40% for *N. incompta* and *G. truncatulinoides*, respectively.

v. Finally, correlations with environmental parameters and GAM indicate that Optimum Growth Conditions, Sea Surface Temperatures and $CO_3^{2-}$ concentration are the most likely parameters influencing planktic foraminifera calcification in the Northwestern Mediterranean. However, calcification appeared to be species-specific and vary depending on the

1238 time scale studied. This may suggest that other parameters than the ones
1239 studied here may play a role in foraminifera calcification.

1240 As planktic foraminifera represent roughly about 50% of pelagic calcite production
1241 (Schiebel, 2002) in the world's oceans, and therefore, an important component of
1242 the marine carbon cycle, a reduction in the calcification of their shell could induce
1243 important changes in the future carbon cycle with feed-backs on climate. Our results
1244 call for increasing efforts in monitoring planktic foraminifera calcification the
1245 Mediterranean in order to determine if the trends suggested by our data will be
1246 sustained over time.

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1251 *Author contributions.* ASRH, FJS and TMB designed the study. JPT designed Figures 1 and
1252 2 and contributed to data discussion. XDM provided Planier core-top and Lacaze-Duthiers
1253 seabed sediment samples. IC provided the Minorca promontory seabed sediment samples.
1254 NH carried out the $^{14}C$ measurements. AH performed the numerical analyses and
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