Holothurians (sea cucumbers) are commonly found in coastal areas, and are well known as detritus feeders and as part of the megabenthos, but there have been few studies on their gut contents. Therefore, a number of holothurians from coastal regions in the Pacific Ocean and Mediterranean Sea were investigated. In addition to lithic particles, the anterior part of the holothurian intestines contained both calcareous (e.g. coccoliths, foraminifera, ostracods), chitinous (e.g. copepod tests) and siliceous (e.g. diatoms, silicoflagellates, sponge spicules) biogenic material, suggesting that holothurians do not discriminate when ingesting small sediment particles. Somewhat surprisingly, however, the coccolith assemblages are composed of taxa representing a wide range of stratigraphic ages (Jurassic–Quaternary), the older presumably eroded from sea cliffs, or brought to the coast from inland outcrops by streams and rivers. In contrast, the posterior part of the intestines contained only siliceous components, suggesting that acidic digestive fluids had dissolved the calcareous and chitinous components. This selective preservation of biogenic particles in holothurian guts may directly lead to an increase in the siliceous content of coastal surface sediments.

Keywords Dissolution, gut contents, holothurians, reworking, selective preservation

1. Introduction
Rivers are thought to be responsible for about 80% of the dissolved silica that enters the world’s oceans (Tréguer et al., 1995), while 80–85% of the organic carbon in marine sediments is sequestered along continental shelves and estuaries (Muller-Karger et al., 2005). This high biological productivity in turn supports a diverse benthic community of filter and detritus feeders, with the feeding activity or burrowing of the latter (e.g. worms, shrimps, holothurians, gastropods) largely being responsible for the bioturbation of surface sediments. Holothurians feed on organic matter, particularly microbial biomass (e.g. Hunt, 1925; Tanaka, 1958; Moriarty, 1982; Sorokin, 1995; Uthicke, 1999), and can be cultured using only diatoms as a food source (e.g. Noguchi & Noda, 2011), but whether or not they are selective feeders is still debatable (see Hammond, 1982). It has been reported that, when in high numbers, the entire layer of surface sediments can pass through their guts in one month, while their slightly acidic (pH ~ 6.7) digestive fluids will dissolve about 2–4g of carbonate sediment per day (Sorokin, 1995), or an average dissolution rate of CaCO₃ in the holothurian gut of 0.41–1.99 mmol per day (Schneider et al., 2011). If true, one would not expect calcareous microfossils to survive passage through holothurian guts, since it is already known that organic contents are significantly higher in the foregut than in the hindgut (16–34% higher for organic carbon, 35–111% for organic nitrogen), due to subsequent digestion and assimilation (Moriarty, 1982).

Therefore, the aims of this study were to investigate holothurian gut contents to determine what they were eating and whether the digestive process affected the biogenic particle composition of the ingested material.

2. Materials and method
2.1 Collection of holothurians
The holothurians were collected from the following four sampling points: 1) Holothuria forskali from Peyrefite Bay, France, in a partially protected area of the Cerbère-Banyuls Marine Protected Area, near the France–Spain
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border, Mediterranean Sea; 2) *H. leucospilota* from Taketomi Island, southern part of the Ryukyu Island Arc, Japan; 3) *H. atra* from the southern part of Okinawa Main Island, Japan; and 4) *Bohadschia marmorata* from Nuku Hiva, Marquesas Islands, Polynesia, central Pacific. Figure 1 shows the locations, Table 1 the sample logistics.

### 2.2 Extraction of gut contents

First, an incision was made along the median line of the holothurian, which connects the mouth and vent (see Appendix 1, Fig. A1). Second, the gastrointestinal tract was removed and separated at approximately the halfway point into anterior and posterior parts, with the anterior part identified by its tentacles or calcareous ring, and the posterior part being on the opposite side of the respiratory tree. In order to retain both calcareous and siliceous components in the gut contents, a modified method, using a domestic pipe-cleaner, was chosen (for more details see Tsutsui & Jordan, 2018). After extraction, each part of the intestine was soaked in 10% density pipe-cleaner fluid (highly condensed type of Lion Look Pipe Man), which contains 0.8% sodium hypochlorite and sodium hydroxide – the lowest density among similar products. The soaked intestine was maintained in a warm water bath at c.50°C in

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**Figure 1**: Holothurian sampling locations. a. Peyrefite Bay, France, Mediterranean. b. Okinawa Main Island, Okinawa Islands, Japan, SW Pacific. c. Taketomi-jima, Yaeyama Islands, Japan, SW Pacific. d. Nuku Hiva, Marquesas Islands, Polynesia, central Pacific. Maps produced by Collaborative Research Center (Sonderforschungsbereich) 574 (http://sfb574.geomar.de/gmt-maps.html)
Coccolith assemblages from holothurian gastrointestinal tracts

a 50cc centrifuge tube for a minimum of 10 minutes to a maximum of 5 hours, depending on the degree of intestine tissue dissolution. At the end of the soaking process, the clear liquid at the top of the centrifuge tube was discarded and replaced with distilled water. This process was repeated two or three times. The material in the centrifuge tube was treated in two ways: 1) the large lithic particles were separated, using a 64µm-mesh sieve (see Appendix 1, Figs A2, A3), and then slides of the microfossils were made from the supernatant; and 2) the large lithic particles were separated using a 32µm-mesh sieve, and then slides of the microfossils (e.g. coccoliths) were made from the supernatant (see Plates 1, 2). Several drops of this liquid were pipetted onto a coverslip and heated on a hotplate at 50˚C until dry. The dried coverslip was temporarily mounted using light-microscope (LM) immersion oil (Olympus IMMOIL-50). The biogenic particles (notably coccoliths) were observed under an Olympus BX-40 LM with a 100x oil-immersion objective, phase contrast illumination and an Olympus U-ANT polariser. They were photographed using a Canon EOS Kiss 6Xi camera with a Micronet NY-1S relay lens. Images were also taken in a JEOL JSM-6510LV scanning electron microscope (SEM). Some of the diatoms, silicoflagellates and sponge spicules found inside the guts of the sea cucumbers are illustrated in Tsutsui & Jordan (2018).

### Results and Discussion

The pipe-cleaner method appeared to have little or no adverse effect on the preservation of coccoliths or other mineralised components (Tsutsui & Jordan, 2018). The common coccoliths found in the anterior intestines of the holothurians in this study are shown in Plates 1 (Peyrefite Bay) and 2 (Okinawa Main Island, Taketomi Island and Nuku Hiva). There were no coccoliths or copepod tests in the posterior parts of the holothurian intestines, although siliceous particles remained, suggesting that the slightly acidic conditions in the foregut caused the dissolution (digestion and possible assimilation) of calcareous and chitinous components. Thus, in their natural habitat, the holothurians may be excreting dissolved carbonate, in addition to siliceous particles, with the alkaline excretion possibly helping to balance the effects of ocean acidification in coral reef areas (Schneider et al., 2011), and making the carbonate ions needed to build coral exoskeletons available. Alternatively, it is possible that the holothurians retain the dissolved carbonate in order to make their calcareous ossicles. Either way, given the vast number of holothurians living in shallow coastal waters (sometimes up to 95 individuals/100m²; Hasan 2005), this constant process of calcium carbonate dissolution in their guts could be quite significant, and have a drastic effect on sediment composition in coastal areas.

Because the coccoliths in the holothurian foreguts from each site represent a wide range of stratigraphic ages (see Fig. 2), and reworked coccoliths can be useful for tracing erosion-transport mechanisms (Ferreira et al., 2008), the possible sources of these coccoliths are discussed below. Somewhat surprisingly, we did not encounter specimens of *Emiliania huxleyi* or small placoliths at any of the sites, suggesting that these coccoliths are either quickly dissolved in the acidic foregut or are rare/in absent from

### Table 1: Sample logistics

<table>
<thead>
<tr>
<th>Holothurian species</th>
<th>Location</th>
<th>Water depth</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Holothuria forskali</em></td>
<td>Peyrefite Bay, France, Mediterranean Sea (42.46°N, 3.15°E)</td>
<td>11.9 m</td>
<td>11 Oct., 2015</td>
</tr>
<tr>
<td>2. <em>H. atra</em></td>
<td>Yaese-cho, Shimajiri, Okinawa (Main Island), Japan, Pacific Ocean (26.13°N, 127.77°E)</td>
<td>0.5 m</td>
<td>12 Oct., 2015</td>
</tr>
<tr>
<td>3. <em>H. leucospilota</em></td>
<td>Kondoi Beach, Taketomi Island, Yaeyama Islands, Japan, Pacific Ocean (24.32°N, 124.07°E)</td>
<td>0.2 m</td>
<td>20 June, 2015</td>
</tr>
<tr>
<td>4. <em>Bohadoschia marmorata</em></td>
<td>Ha’a o Tupa, Nuku Hiva, Marquesas Islands, Polynesia, Pacific Ocean (8.56°S, 140.06°W)</td>
<td>ca. &lt;15 m</td>
<td>25 Nov., 2015</td>
</tr>
</tbody>
</table>
Plate 1

Nannofossils from the anterior intestine of *Holothuria forskali*, Peyrefite Bay, western Mediterranean

1. *Reticulofenestra bisecta*
2. *Reticulofenestra perplexa*
3. *Reticulofenestra haqii*
4. indeterminate placolith

5. *Watznaueria britannica*
6. *Watznaueria britannica*
7. *Watznaueria biporta?*
8. *Watznaueria manivitiae*

9. *Watznaueria barnesiae*
10. *Lotharingius contractus?*
11. *Watznaueria manivitiae*
12. *Markalus inversus?*

13. *Helicosphaera carteri*
14. *Helicosphaera ethologa?/carteri group*
15. *Scyphosphaera apsteinii*
16. *Scyphosphaera apsteinii*

17. *Pontosphaera exilis*
18. *Eiffellithus turriseiffelii*
19. *Zeugrhabdotus erectus?*
20. *Zeugrhabdotus erectus?*

21. *Biscutum ellipticum*
22. *Diaziomatolithus lehmanii?*
23. indeterminate cretaphid?*
24. *Retecapsa* sp.

25. indeterminate placolith
26. *Braarudosphaera bigelowii*
27. *Ceratolithus cristatus*
28. *C. cristatus*

29. *Dictyocha* sp.
30. *Corbisema* sp.
**Plate 2**

Nannofossils from the anterior intestine of *Holothuria atra*, Main Island, Okinawa, western Pacific

1. *Reticulofenestra pseudoumbilicus*
2. *R. pseudoumbilicus*
3. *Coccolithus pelagicus*
4. *Calcicidiscus tropicus*

5. *Helicosphaera carteri* group
6. *Braarudosphaera bigelowii*
7. *Discoaster surculus*

Nannofossils from the anterior intestine of *H. leucospilota*, Kondoi Beach, Taketomi Island, western Pacific

8. *Reticulofenestra haqii*
9. *Reticulofenestra minuta*
10. *Reticulofenestra cisnerosii*?
11. *Gephyrocapsa* sp.

12. *Cyclagelesphera deflandrei*
13. indeterminate placolith?
14. indeterminate placolith

Nannofossils from the anterior intestine of *Bohadschia marmorata*, Ha’a o Tupa, Nuku Hiva, western Pacific

15. *Gephyrocapsa* sp.
16. *R. minuta*
17. *R. minuta*
18. *R. minuta*

19. indeterminate placolith
20. *Reticapsa angustiforata*
21. *Calcicidiscus leptoporus*
22. *Calculites obscurus*?

23. *Uniplanarius gothicus*?
24. *Uniplanarius gothicus*?
25. indeterminate coccolith
26. *H. carteri*

27. *Calciosolenia murrayi*
28. ascidian spicule
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these shallow coastal waters.

**Peyrefitte Bay, France**

The foregut of *H. forskali* contained reworked coccoliths of Cenozoic and Mesozoic taxa, some of them restricted to the Jurassic. There are numerous rivers that transport sediments and discharge them into the Gulf of Lions. Excluding those that flow from the metamorphic Pyrénées and the volcanic formations of the Massif Central, the Rhône River has the largest discharge volume of all the rivers in this region, and it is known that sediments in the Gulf of Lions mainly originated from its watershed (Revillon et al., 2011). The Rhône is known to erode Alpine sedimentary rocks as old as the Jurassic in its upper catchment in the Jura Mountains (Sommaruga, 1997; de Graciansky et al., 1998; Trümpy, 1980), transporting material southwards via Lake Geneva. Jurassic rocks are also cut by two of the Rhône’s tributaries – the Ardèche in its southern catchment and the Saone on its way to Lyon. Younger coccoliths are also likely to come from the Rhône, although the Pyrénées Mountains (Cretaceous and Cenozoic) and the Aquitanian Basin (Paleogene and Neogene) may be alternative source areas. Inside the Gulf, a southerly wind (the Marin) drives the surface currents that transport the discharged material along the coast towards the French–Spanish border at Cap de Creus (Palanques et al., 2006). In contrast, the diatoms appear to be represented only by extant taxa (e.g. *Diploneis*, *Surirella*; data not shown here), although the silicoflagellates (*Corbisema* sp. and *Dictyocha* sp.) also appear to be much older.
Main Island, Okinawa
The presence of reworked (extinct) coccoliths and silicoflagellates in the guts of the Okinawa and Taketomi holothurians suggests that an outcrop of older material is being eroded and incorporated into the modern coastal sediments. On Okinawa and Taketomi Islands, the Ryukyu Limestone (Lower to Middle Pleistocene; e.g. Yabe & Hanzawa, 1930; MacNeil, 1960; Omura, 1988; Ujié, 1994; Iryu et al., 2006) overlies the Shimajiri Group (Miocene to Pliocene; e.g. Nishida, 1980; Tanaka & Ujié, 1984; Sato, 2004; Odawara et al., 2006). Thus, the finding of Discoaster surculus in both holothurians may not be so surprising.

Taketomi, Yaeyama Islands
Cyclagelosphaera became extinct in the Early Eocene; however, the recent rediscovery of living Tergestiella adriatica, with its almost identical morphology to Cyclagelosphaera (Hagino et al., 2015), suggests that the latter genus may have survived into the Neogene and beyond. This, together with the finding of Reticulofenestra spp. in the holothurian gut, suggests a Miocene–Pliocene age. The Fusaki Formation of the Ishigaki Group (pre-Eocene) crops out on Taketomi, which is largely covered in Ryukyu Limestone of Pleistocene age (Nakagawa et al., 1982), while sediments range in age from Paleogene to Pleistocene on the nearby Ishigaki Island (Foster, 1965; Nakagawa et al., 1982).

Nuku Hiva, Marquesas Islands
Today, a low-diversity coastal coccolithophorid assemblage can be found around the island (see Jordan & Riaux-Gobin, 2019 [this issue]); however, many of the nannofossils in the holothurian foregut are clearly reworked (either bioturbated or from uplifted/eroded sediments), and are either younger or much older (e.g. Jurassic and Cretaceous taxa, such as Retecapsa, Uniplanarius and Calculites) than the shield volcano of Nuku Hiva, which developed between 4.8 and 3.7Ma, with a caldera collapse event around 4.05Ma (Le Dez et al., 1996). The basaltic rocks of the oceanic crust should not be older than the Lower Paleocene, however (Kruse, 1988), which means that Mesozoic sediments are unlikely to have been incorporated by the uplifting lavas during eruptions and volcanic island construction (besides, the high temperature is likely to have recrystallised the coccoliths; however, see Zaczek et al., 2015). Thus, at present, we cannot explain the existence of the older coccoliths in the foregut of B. marmorata.

4. Summary
Four holothurian species (H. forskali, H. atra, H. leucospilota and B. marmorata) were collected from the Mediterranean Sea and western and central Pacific Ocean. Coccoliths from a wide range of geological ages (Jurassic–Quaternary), including coastal species, were found in the holothurians’ anterior intestines. In the case of Peyrefitte Bay, there was a clear association between the coccolith assemblage in the holothurian foregut and that likely to be found in the upper catchment area of the Rhône River (i.e. the Alps); however, unlike the siliceous component (e.g. diatoms, sponge spicules, silicoflagellates), coccoliths were absent from the posterior intestines. We can conclude that: 1) holothurians are indiscriminate detritus feeders on <10mm sediment/food particles; 2) coccoliths are dissolved by acidic holothurian digestive fluids; and 3) holothurians play a role in determining the coastal sediment composition.

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Appendix 1
Holothurian anatomy and example of gut contents

A1. Anatomical structure of a *H. atra* specimen from Main Island, Okinawa. Anterior (left) and posterior (right); respiratory tree cannot be seen because it lies beneath the digestive tract. The tentacles are also obscured.

A2. Foregut content of specimen in Figure A1, showing particles >63µm in size.

A3. Hindgut contents of specimen in Figure A1, showing particles >63µm in size.