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*Minireview*

**Microbial Trimethylamine Metabolism in Marine  
Environments**

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**Running title:** Microbial TMA metabolism

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**Originality-Significance Statement:** We identify the key aspects of originality and  
significance that place the work within the top 10% of current research in  
environmental microbiology.

22 **Summary:**

23       Trimethylamine (TMA) is common in marine environments. Although the  
24 presence of this compound in the oceans has been known for a long time, unlike the  
25 mammalian gastrointestinal tract, where TMA metabolism by microorganisms has been  
26 studied intensely, many questions remain unanswered about the microbial metabolism  
27 of marine TMA. This mini-review summarizes what is currently known about the  
28 sources and fate of TMA in marine environments and the different pathways and  
29 enzymes involved in TMA metabolism in marine bacteria. This review also raises  
30 several questions about microbial TMA metabolism in the marine environments, and  
31 proposes potential directions for future studies.

## Introduction

32

33       Trimethylamine (TMA) is one of the several methylated amines that are ubiquitous  
34 in marine systems (King, 1984a; Gibb et al., 1999a; Gibb and Hatton, 2004) and make  
35 up an important component of the oceanic carbon and nitrogen pools (Gibb and Hatton,  
36 2004; Chen *et al.*, 2011; Carpenter *et al.*, 2012) (Figure. 1). TMA has the characteristic  
37 odor of rotting fish. It was found to be produced during fish spoilage in the 1930s  
38 (Beatty, 1938) and later was recognized as a malodorous pollutant (Sandberg and  
39 Ahring, 1992; Rappert and Muller, 2005). TMA is a precursor of other methylated  
40 amines, for example trimethylamine *N*-oxide (TMAO), through an oxidation pathway  
41 that will be explained further down. TMAO is a common osmolyte used by many  
42 marine biota to regulate osmotic pressure and stabilize proteins against denaturation  
43 (Barrett and Kwan, 1985; Seibel and Walsh, 2002).

44       TMA first attracted the interest of biogeochemists because it is an important  
45 precursor for methane formation in a variety of marine environments (Figure. 1). Under  
46 anoxic conditions, up to 90% of the methane from salt marsh sediment or slurries can  
47 be attributed to microbial conversion of TMA from the degradation of quaternary amine  
48 precursors (Oremland et al., 1982). A similar finding by an independent research group  
49 also found that 35 to 61% of total methane in surface sediments of an intertidal mud  
50 flat could result from TMA metabolism (King *et al.*, 1983, King GM 1984a). TMA is a  
51 component of marine aerosols. Along with other methylated amines (*e.g.*  
52 dimethylamine (DMA) and monomethylamine (MMA)), it is emitted from surface  
53 seawater into the atmosphere, which can actively affect the climate system (Carpenter

54 *et al.*, 2012; Lidbury *et al.*, 2017) (Figure.1).

55         Currently, there are two major challenges for biogeochemists in the study of  
56 oceanic methylated amines: the first is to identify the major sources of these compounds  
57 - what is the source of marine TMA? The second challenge concerns the fate of this  
58 compound - who are the major TMA consumers in marine environments? In this review,  
59 we first summarize what is known about the distribution of oceanic TMA and discuss  
60 the significance and sources of this compound in marine environments. We then  
61 describe three different metabolic pathways of TMA degradation and the enzymes that  
62 catalyze the reactions. Finally, we discuss the potential issues and challenges in the  
63 study of TMA metabolism and conclude by highlighting opportunities for future  
64 research directions.

65

## 66                                   **Sources of marine TMA**

67         It was not until the 1990s, when highly sensitive analytical techniques became  
68 available for the measurement of methylated amines, that researchers gained the  
69 capability to quantify TMA in the oceans reliably and accurately. Ocean-scale research  
70 revealed that concentrations of TMA range from nanomolar (nM) to micromolar ( $\mu$ M),  
71 depending on the marine environment sampled (Table 1). In surface water, TMA  
72 concentrations are low. Unlike DMA and MMA, TMA concentrations in seawater had  
73 no seasonal pattern and did not correlate with the abundance of diatoms nor  
74 mesozooplankton grazing activities (Gibb *et al.*, 1999b). However, methylated amines  
75 can be strongly adsorbed to marine sediments, particularly to those with a high organic

76 content (Wang and Lee, 1990), which may help to explain the elevated TMA  
77 concentrations observed in marine sediments (Table 1).

78         Although the importance of TMA in the global carbon and nitrogen cycle is being  
79 recognized (Lee and Olson, 1984; Gibb and Hatton, 2004; Chen et al., 2011), the  
80 sources of this compound in marine ecosystems are not well established. Many marine  
81 plants and animals have been found to contain high concentrations of methylated  
82 amines (Wang and Lee, 1994; Calderón *et al.*, 2007). Hence, one hypothesis is that  
83 TMA is directly released from tissues during excretion or decay of marine organisms.  
84 It has been reported that TMA is commonly found in marine algae (Fujiwara-Arasaki  
85 and Mino, 1972; Smith, 1975) and TMA production is associated with annual  
86 senescence and production of marsh grass in salt marsh sediments (Wang and Lee,  
87 1990). Wang and Lee experimentally demonstrated that the plant *Spartina alterniflora*  
88 gets decomposed to release amines, especially TMA, to salt marsh sediments (Wang  
89 and Lee, 1994). Fish, benthic animals and phytoplankton also contain high  
90 concentrations of methylated amines and could be important sources of oceanic TMA,  
91 either by direct release or through decomposition (Shewan, 1951; Budd and Spencer,  
92 1968; Barrett and Kwan, 1985; Wang and Lee, 1994).

93         Another potential source of TMA is degradative pathways that form TMA as an  
94 intermediate or end-product. Potential TMA sources include common organic  
95 compounds such as the compatible solutes (osmolytes) TMAO, glycine betaine, and  
96 choline, which are abundant in marine eukaryotic cells (Ikawa and Taylor, 1973; King,  
97 1984a; Oren, 1990; López-Caballero et al., 2001; Treberg et al., 2006). These

98 compounds can be transformed to produce TMA by a TMAO reductase (TorA), a  
99 glycine betaine reductase (GrdH) or a choline-TMA lyase (CutC), respectively.  
100 Metagenomic studies have shown that the *grdH* gene is present in marine environments  
101 but at low abundance. The *cutC* gene is more prevalent in anaerobic marine sediments.  
102 Among these functional genes, the *torA* gene is the most abundant in both open ocean  
103 and marine sediment datasets, which implies that TMA formation from the TMAO  
104 reduction pathway is prevalent and important in the oceans (Jameson *et al.*, 2016).

105 TMA production can also occur under aerobic conditions through oxidation of  
106 carnitine (Unemoto *et al.*, 1966; Rebouche and Seim, 1998; Zhu *et al.*, 2014), which  
107 may explain the presence of TMA in oxygenated marine surface waters (Carpenter *et al.*,  
108 2012). Notably, but rarely studied, TMA can be produced from the betaine-  
109 containing lipid diacylglyceryl hydroxymethyl *N,N,N*-trimethyl- $\beta$ -alanine (DGTA) by  
110 a spontaneous deamination process (Vogel *et al.*, 1990). DGTA is widely distributed in  
111 marine phytoplankton (Araki *et al.*, 1991; Cañavate *et al.*, 2016).

## 112 **The metabolic fate of marine TMA**

113 The study of TMA metabolism has been primarily focused on methylotrophic  
114 bacteria and methanogens (Hippe *et al.*, 1979). These microorganisms can use  
115 methylated amines as their carbon, nitrogen and energy sources (Chistoserdova *et al.*,  
116 2009; Chistoserdova, 2011). Generally, there are four different pathways for microbial  
117 metabolism of TMA: acetogenesis pathway, methanogenesis pathway, the  
118 dehydrogenase pathway and the aerobic oxidation pathway. The bacterium  
119 *Acetohalobium* is capable of demethylating TMA to an equimolar amount of acetate

120 along with less amounts of DMA and MMA via anaerobic acetogenesis (Zhilina and  
121 Zavarzin, 1990), although not much is known on the genes/enzymes involved. TMA-  
122 dependent acetogenesis has been rarely studied; therefore we will mainly describe the  
123 other three pathways, which are depicted in Figure 2.

124

### 125 **TMA-dependent methanogenesis**

126 A number of investigations have shown that TMA can be a significant source of  
127 methane in a variety of marine systems (Oremland *et al.*, 1982; Oremland and Polcin,  
128 1982; King *et al.*, 1983; Summons *et al.*, 1998). Oremland *et al.*, and King *et al.*,  
129 showed that the addition of TMA to marine sediments stimulates the production of  
130 methane (Oremland *et al.*, 1982; King *et al.*, 1983; King, 1984b). Several novel strains  
131 of methylotrophic methanogens have been isolated from anoxic marine sediments  
132 which can catabolize TMA to produce methane (Singh *et al.*, 2005). Although the  
133 phenomenon of methanogenesis from TMA has been observed for many years in anoxic  
134 oceans (Hippe *et al.*, 1979; Sowers *et al.*, 1984; Siegert *et al.*, 2011), the featured species  
135 and metabolic process in marine environments are still unclear.

136 Notably, in similar anaerobic environments, such as the gastrointestinal tract of  
137 ruminants and sewage sludge digesters, TMA has been shown to be a significant  
138 substrate for methylotrophic methanogens (Neill *et al.*, 1978; Mah and Kuhn, 1984;  
139 Zinder *et al.*, 1985; Zhilina and Zavarzin, 1987). Methanogenesis is a metabolic process  
140 driven by obligate anaerobic *Archaea*. Methanogens, such as members of the  
141 order *Methanomassiliicoccales* and *Methanosarcinales*, can use methyl groups from



142 TMA to firstly produce methyl-coenzyme M (methyl-CoM) by the concerted action of  
143 two methyltransferases: TMA methyltransferase and coenzyme M methyltransferase  
144 (Ferguson and Krzycki, 1997; Bose et al., 2008). Methyl-CoM is subsequently  
145 converted into methane, CO<sub>2</sub> and ammonia by a methyl-CoM reductase (MCR), the  
146 key enzyme of methanogenesis (Figure 2A) (Friedrich, 2005; Kröninger et al., 2017).

147 Recent bioinformatics analyses of metagenomes and metatranscriptomes provided  
148 further evidence of the TMA-dependent methanogenesis pathway in marine  
149 environments. For example, the alpha-subunit of MCR (*mcrA*) was detected from  
150 sediment samples of the Western Mediterranean Sea by PCR amplification.  
151 Phylogenetic analysis revealed the presence of diverse methanogen communities  
152 distributed along the different geochemical zonations, including those from known  
153 TMA-utilizers e.g. *Methanococoides* and *Methanosarcina* (Zhuang et al., 2018).  
154 Similarly, metatranscriptomic data from anoxic sediment in the Baltic Sea revealed that  
155 *mcrA* transcripts affiliated to *Methanosarcina* were highly abundant, suggesting a role  
156 of TMA-dependent methanogenesis in the sediment (Thureborn et al., 2016).

157

### 158 **Anaerobic TMA dehydrogenase pathway**

159 The second pathway of TMA degradation involves the direct dehydrogenation of  
160 TMA to form DMA and formaldehyde, catalyzed by a TMA dehydrogenase (TMADH)  
161 (Colby and Zatman, 1973; Kasprzak et al., 1983; Yang et al., 1995). In some  
162 methylotrophs, DMA is further demethylated to MMA and then ammonia by a series  
163 of dehydrogenase enzymes: DMA dehydrogenase (DMADH) and MMA

164 dehydrogenase (MMADH), with each step simultaneously forming the side-product  
165 formaldehyde (Figure 2B) (Asatoor and Simeshoff, 1965; Colby and Zatman, 1973;  
166 Barrett and Kwan, 1985; Chistoserdova, 2011). The whole pathway is energetically  
167 favorable and oxygen is not required for these processes. However, this energy-saving  
168 pathway seems not to be important in marine microorganisms, since little evidence for  
169 these dehydrogenases have been found in marine metagenomic data. Instead, pathways  
170 for aerobic TMA degradation by bacterioplankton, which are discussed below, have  
171 been intensively studied.

172

### 173 **Aerobic TMA oxidation pathway**

174 This pathway involves the oxygenation of TMA to TMAO, which is further  
175 catabolized to DMA, MMA, ammonia and formaldehyde (Figure 2C). The initial step  
176 of conversion of TMA to TMAO is mediated by a TMA monooxygenase (Tmm). Tmm  
177 is a flavin-dependent enzyme. Bacterial Tmm was first identified and characterized in  
178 the soil bacterium *Methylocella silvestris* (Dunfield et al., 2003; Chen et al., 2011).  
179 Enzymatic activity assays showed that the marine *Roseobacter* clade (*Roseovarius* sp.  
180 217 and *Ruegeria pomeroyi* DSS-3) and SAR11 clade (HTCC1002 and HTCC7211),  
181 two of the most abundant bacterioplankton groups in the surface ocean, also have Tmm  
182 enzymes to catabolize TMA oxidation (Chen *et al.*, 2011). Metagenomic evidence  
183 revealed that most marine bacterioplankton possess TMA monooxygenase, leading to  
184 the estimate that about 20% of the bacteria in the surface ocean contain this gene (Chen  
185 *et al.*, 2011). This suggests that aerobic TMA degradation is the major pathway for

186 TMA utilization in the marine environment, especially in the oxygen-rich surface water.

187 Most recently, the molecular mechanism of TMA oxygenation by marine bacterial  
188 Tmm was elucidated (Li *et al.*, 2016). There are two half-reactions (reductive and  
189 oxidative) in the catalytic process. In the first half-reaction, flavin adenine dinucleotide  
190 (FAD) is reduced by nicotinamide adenine dinucleotide phosphate (NADPH), and an  
191 intermediate C4a-hydroperoxyflavin is formed. In the second half-reaction, this  
192 intermediate attracts TMA to the catalytic pocket. TMA binding to the catalytic site of  
193 Tmm causes a conformational change in NADP<sup>+</sup>, which shuts off the substrate entrance  
194 and exposes C4a-hydroperoxyflavin to TMA, thereby starting the oxidative half-  
195 reaction (Li *et al.*, 2016).

196 After oxidation, the oxygenated form, TMAO, is further demethylated to yield  
197 DMA and formaldehyde by a TMAO demethylase (Tdm) (Chen *et al.*, 2011; Lidbury  
198 *et al.*, 2014; Lidbury *et al.*, 2015). Tdm was first proposed and partially purified from  
199 *Bacillus* (Myers and Zatman, 1971) and methylotrophs such as *Pseudomonas*  
200 *aminovorans* (Large, 1971; Boulton *et al.*, 1974) and *Hyphomicrobium* spp. (Meiberg  
201 *et al.*, 1980; Barrett and Kwan, 1985). Recently, Tdm has been demonstrated to occur  
202 in abundant marine heterotrophic bacteria as well (Chen *et al.*, 2011; Lidbury *et al.*,  
203 2014; Lidbury *et al.*, 2015). Although the enzyme can be purified from aerobic bacteria,  
204 Tdm is oxygen-independent and is not affected in aerobic or anaerobic conditions  
205 (Large, 1971). This enzyme is strongly activated by Zn<sup>2+</sup> and Fe<sup>2+</sup> metal cofactors (Zhu  
206 *et al.*, 2016).

207 Conversion of DMA to MMA by a secondary amine monooxygenase has been

208 proposed for a long while (Alberta and Dawson, 1987; Alberta *et al.*, 1989). The  
209 enzymology of this protein was also first characterized from *P. aminovorans* by  
210 spectroscopic analysis (Alberta *et al.*, 1989) and was later known as a heme-dependent  
211 oxidative *N*-demethylase with a heme-dependent Per-ARNT-Sim (PAS)-domain  
212 (Ortmayer *et al.*, 2016). This particular PAS enzyme is a heterotetramer, and requires  
213 NADPH in the DMA catabolic pathway (Ortmayer *et al.*, 2016) .

214       Only recently did a study confirm that the gene *dmmDABC* encodes a functional  
215 DMA monooxygenase (Dmm) in *R. pomeroyi* DSS-3 for DMA demethylation (Lidbury  
216 *et al.*, 2017), which fills a gap and completes the marine DMA degradation pathway.  
217 The genes encoding DmmDABC are widely distributed in the marine *Roseobacter*  
218 clade, whereas they are absent from the genomes of some important marine bacterial  
219 taxa, including all representatives of the SAR11 clade. This would explain why the  
220 abundance of the gene cluster *dmmDABC* was much lower in marine metagenomics  
221 data than the other relative genes involved in degradation of methylated amines  
222 (Lidbury *et al.*, 2017).

### 223                                   **Concluding remarks and future prospects**

224       Although the significance of marine TMA is recognized, the sources, fluxes and  
225 fates of this compound in the ocean are still not fully understood. The development of  
226 better analytical methods for the *in situ* quantification of methylated amines remains a  
227 challenging problem (Lee and Olson, 1984; Abdul-Rashid *et al.*, 1991; Yang *et al.*,  
228 1993). A recent improvement by Zhuang *et al.*, (2017) used a method combining a purge  
229 and trap system coupled with gas chromatography-mass spectrometry (P&T-GC-MS).

230 This method quantifies TMA in one analytical step, requires small volumes (5 mL) of  
231 porewater or sediment samples, and can simultaneously measure the stable carbon  
232 isotopic composition in the solid phase of marine sediments (Zhuang *et al.*, 2017). More  
233 recently, Cree *et al.* reported another method to determine dissolved methylated amines  
234 in seawater samples. Methylated amines converted to the gaseous phase were analyzed  
235 by coupling headspace solid phase microextraction (SPME) and gas chromatography  
236 coupled with a nitrogen–phosphorus detector (GC-NPD) (Cree *et al.*, 2018). This  
237 method provides lower detection limits and is more suitable for measuring methylated  
238 amines at low-nM level in marine environments. Compared to the P&T-GC-MS system,  
239 SPME-GC-NPD has better sensitivity to the low-molecular weight amines, but requires  
240 a larger sampling volume (1L). During the SPME extraction process, maintaining the  
241 thermostat and homogeneity of seawater samples is particularly important. Although  
242 keeping the equilibrium of one sample in the study is available, operating parallel  
243 extractions from multiple large volume samples under the same conditions may be  
244 difficult to control. The possible solution would be to combine the purge and trap  
245 system with the SPME extraction, which could create a constant equilibrium between  
246 aqueous phase and gaseous phase with less interference from temperature variations. In  
247 addition, the introduction of inert gas flow could potentially improve the recoveries of  
248 methylated amines to achieve a better sensitivity and more accurate measurements.  
249 With the development in methodology, more information on *in situ* concentrations of  
250 methylated amines is likely to become available in the near future, contributing to a  
251 better understanding of TMA biogeochemistry.

252 In nature, some microorganisms have been found to possess pathways for both the  
253 aerobic and anaerobic degradation of TMA. This raises two questions: why do some  
254 microbes require two metabolic pathways, and are these two pathways independent or  
255 related? *Paracoccus* sp. Strain T231 can use two different enzymes, Tmm and TMADH,  
256 to initialize the degradation of TMA in aerobic and anaerobic metabolism, respectively  
257 (Kim *et al.*, 2001). When grown aerobically on TMA, enzyme activities of Tmm, Tdm,  
258 Dmm and MMA monooxygenase from cell-free extract are detected. When grown  
259 anaerobically on TMA and nitrate, enzyme activities of TMADH and DMADH from  
260 the cell-free extract are detected (Kim *et al.*, 2001). In contrast, in aerobic metabolism,  
261 both Tmm and TMADH can be used to initialize the oxidation of TMA in *Pseudomonas*  
262 *putida* ATCC 12633 (Liffourrena *et al.*, 2010).

263 TMA metabolism of *Hyphomicrobium* is more complicated. This microorganism  
264 is commonly found in soil and fresh water (Harder and Attwood, 1978) and is able to  
265 oxidize TMA by TMADH under both aerobic and anaerobic conditions in the presence  
266 of nitrate (Meiberg and Harder, 1978). For the two known pathways of DMA  
267 demethylation to MMA, oxygen and TMA availability are the key regulatory factors.  
268 The enzyme Dmm is strictly dependent on oxygen as a substrate. Dmm activity was  
269 undetectable when oxygen was absent in the medium, and was expressed immediately  
270 when oxygen was provided. Although the activity of DMADH is independent of  
271 oxygen, the synthesis of DMADH in *Hyphomicrobium* X was inhibited by high oxygen  
272 tensions, and lowering the oxygen tension relieved this inhibition (Meiberg *et al.*, 1980).  
273 In addition, TMA concentrations were proposed to regulate DMADH activity. During

274 the initial stage of cell growth on TMA, a high concentration of TMA acts as a potent  
275 competitive inhibitor for DMADH, and the product DMA accumulates in the medium.  
276 As TMA is degraded and the concentration decreases, DMADH is upregulated, which  
277 allows for the subsequent catabolism of DMA (Meiberg and Harder, 1979; Meiberg *et*  
278 *al.*, 1980). Overall, these regulatory properties could provide this microorganism with  
279 a selective advantage over competitors in habitats where oxygen and TMA  
280 concentrations fluctuate.

281 In marine environments, knowledge of microbial TMA catabolism is limited to a  
282 few studies. Genome analysis of heterotrophic bacteria that are abundant in marine  
283 surface water (i.e. the *Roseobacter* and SAR11 clade) revealed gene clusters only for  
284 aerobic TMA catabolism and the physiological experiments confirmed oxidative  
285 degradation of TMA via TMAO as the key intermediate (Chen *et al.*, 2011; Sun *et al.*,  
286 2011). Metagenomic data from global ocean sampling have also shown an abundance  
287 of the *tmm* gene and low frequency of *dmm* (Lidbury *et al.*, 2017), implying the  
288 adaptation of dominant plankton groups to oxygen and the significance of the TMA  
289 oxygenation pathway in marine surface water. However, due to the lack of  
290 metagenomic data in hypoxic zones, whether anaerobic TMA degradation occurs under  
291 low oxygen conditions of the water column is still unknown. Up till now, only a few  
292 marine bacterial species, such as a methylotrophic bacterium *Methylophaga* sp. strain  
293 SK1 and some denitrifying bacteria isolated from coastal sediments (Kim *et al.*, 2003),  
294 have been found to contain both TMADH and Tmm metabolic pathways for TMA  
295 degradation (Choi *et al.*, 2003; Kim *et al.*, 2006; Chen *et al.*, 2011). However, the

296 regulation of the anaerobic dehydrogenase pathway and the aerobic TMA oxidation  
297 pathway in these marine microorganisms is poorly characterized, which limits the  
298 understanding of the adaption of these marine bacteria to their surrounding habitats and  
299 their ecological significant. All of these questions remain to be explored, and will likely  
300 be the focus of future research.

301

302

303

#### 304 **Conflict of interest statement**

305 The authors declare that the research was conducted in the absence of any commercial  
306 or financial relationships that could be construed as a potential conflict of interest.

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311 **Tables:**

312 **Table 1. Concentration of TMA in marine environments as reported in the**  
313 **literature**

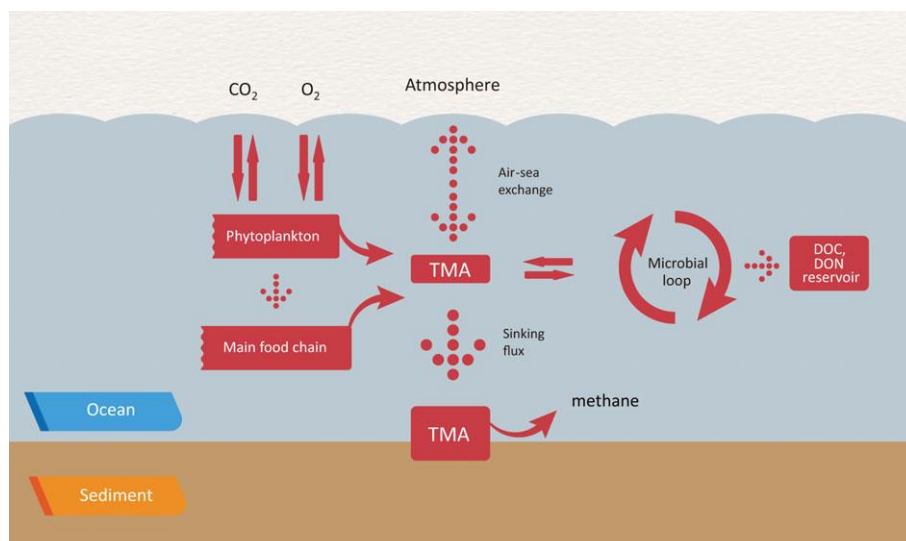
<b>TMA concentration</b>	<b>Source/Location</b>	<b>References</b>
12 ± 3.0 nM	Pacific—Hawaii coastal	(Van Neste <i>et al.</i> , 1987)
41 ± 27 nM	Atlantic—Massachusetts coastal	
1.4 ± 1.6 nM	Offshore, Mediterranean	(Gibb, 1994; Gibb <i>et al.</i> , 1999b)
10 ± 6.9 nM	Costal, Mediterranean	
< 4 nM	Arabian Sea	(Gibb <i>et al.</i> , 1999b)
1.6 ± 1.8 nM	Antarctic coastal waters	(Gibb and Hatton, 2004)
< 3-80 nM	Flax Pond seawater, New York	(Yang <i>et al.</i> , 1993)
20 nM	Western English Channel	(Cree <i>et al.</i> , 2018)
1.4-6.9 nM	Southern Ocean	
0 - 4.7µM	Porewater of East Anglian Estuary sediments	(Fitzsimons <i>et al.</i> , 2001)
0 - 50 µM	Porewater of Oglet Bay sediments	(Fitzsimons <i>et al.</i> , 1997)
0 - 15 µM	Porewater of Norsminde Fjord Estuary sediments	(Glob and Sørensen, 1987)
0.6 µM	Porewater of Flax Pond salt marsh	(Wang and Lee, 1990, 1994)

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317 **Figure legends:**



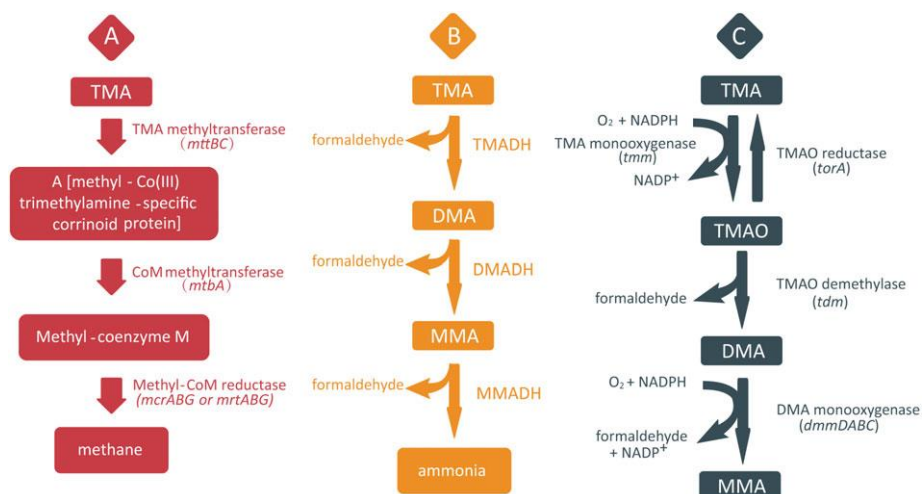
318

319 **Figure 1. Diagram of marine biogeochemical cycles of TMA.** DOC: dissolved

320 organic carbon; DON: dissolved organic nitrogen; TMA: trimethylamine.

321

322



323

324 **Figure 2. Proposed three main TMA metabolic pathways in marine microbes. A)**

325 Methanogenesis; B) Anaerobic TMA dehydrogenase pathway; C) Aerobic TMA

326 oxidation pathway. DMA: dimethylamine; MMA: monomethylamine; TMA:

327 trimethylamine; TMAO: trimethylamine *N*-oxide;  $NADP^+$ : nicotinamide adenine

328 dinucleotide phosphate; NADPH: reduced form of nicotinamide adenine dinucleotide

329 phosphate. TMADH: trimethylamine dehydrogenase; DMADH: dimethylamine

330 dehydrogenase; MMADH: monomethylamine dehydrogenase.

331

332

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