

# 1 **Exceptional accumulation and retention of dimethylsulfoniopropionate by molluscs**

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7

## 8 **Introduction**

9 Many types of marine phytoplankton synthesize dimethylsulfoniopropionate (DMSP),  
10 which yields the climate gas dimethylsulfide (DMS) by a simple cleavage reaction. Ever since  
11 Dacey & Wakeham<sup>[1]</sup> demonstrated that phytoplankton-consuming animals can strongly affect  
12 the rate at which algal DMSP is converted to DMS, biologists have sought to understand the  
13 effects of each of the major phytoplankton-consuming animal groups on DMSP/DMS dynamics.

14 Phytoplankton-consuming molluscs, such as the blue mussel (*Mytilus edulis*), are  
15 potentially major actors in DMSP/DMS dynamics in a variety of ocean settings. This is true  
16 because individuals can remove phytoplankton cells from impressively large volumes of water  
17 per unit of time, and enormous numbers of individuals may be present in an ecosystem. Blue  
18 mussels illustrate these points. At temperatures near 10-20°C, individual 6- to 7-cm-long *M.*  
19 *edulis* pump water at 10-20 L h<sup>-1</sup> through their feeding apparatus when feeding.<sup>[2-4]</sup> As they  
20 process this water, they retain – and later metabolize – essentially 100% of algal cells of 4 μm  
21 diameter or larger, 90% of 3 μm cells, and 50% of 1 μm cells.<sup>[5]</sup> Equally important, *M. edulis*  
22 populations often consist of hundreds of mussels attached to each m<sup>2</sup> of benthic substrate.<sup>[6]</sup>  
23 Riisgård<sup>[6]</sup> calculated that a population of *M. edulis* in Limfjord (Denmark) processed 180 m<sup>3</sup> of

24 ambient water  $\text{m}^{-2} \text{d}^{-1}$ , a rate that Riisgård<sup>[6]</sup> calculated to be equivalent to 20 times the local  
25 water column each day. Mollusc populations dominated by *M. edulis* in parts of the coastal  
26 Wadden Sea are able to clear all phytoplankton from the entire local volume of water in 2-5  
27 days, and they harvest from 18% to >100% of local phytoplankton production.<sup>[7]</sup> Such estimates  
28 suggest that in places like the Wadden Sea, 18% to >100% of local algal DMSP production is  
29 processed first by molluscs. With respect to the open ocean, certainly herbivorous pteropods  
30 (planktonic molluscs) have the potential to be major phytoplankton and DMSP consumers at the  
31 times and places of their blooms.<sup>[8]</sup> In short, there is every reason to believe that molluscs often  
32 process a sizable fraction of local phytoplankton DMSP production, poising them to exert strong  
33 effects on local DMSP/DMS dynamics.

34 In this deliberately brief report, we aim to bring into focus a set of related, basic questions  
35 that have arisen in our research on the physiology of DMS(P) processing in molluscs [by  
36 DMS(P) we mean either DMSP or DMS]. We have studied DMS(P) processing in a variety of  
37 animals, including fish and crustaceans.<sup>[1,9]</sup> From this perspective, it is clear that some molluscs  
38 present unique properties and challenges.

39 Although we will mention the tridacnid clams, which live symbiotically with DMSP-  
40 producing dinoflagellates,<sup>[10,11]</sup> our concern here is chiefly with molluscs that lack algal  
41 symbionts. These molluscs – which constitute the great majority – are thought to acquire all  
42 tissue DMS(P) heterotrophically.

43 The focus of our argument is that some molluscs – after they accumulate DMS(P) from  
44 their foods – seem to retain tissue DMS(P) to an exceptional degree in comparison with other  
45 phyletic groups of animals. This phenomenon has two principal implications. The first is  
46 practical, namely that tight tissue retention can present major obstacles to mass balance studies;

47 we ourselves have had several experiments defeated by tissue retention, leading us to the view  
48 that tight tissue retention is an essential factor to consider in experimental designs. Second, the  
49 tight tissue retention of some molluscs suggests that tissue DMS(P) may be playing functional  
50 roles in molluscs or that DMS(P) might bind relatively tightly to tissue constituents, a  
51 phenomenon that in itself could be of functional importance. In this way, retentiveness – a  
52 phenomenological property – might be pointing to as yet unknown physiological roles for  
53 DMS(P).

54 Few studies on molluscs have been targeted at understanding DMS(P) accumulation and  
55 retention. Instead, most evidence on the subject comes from incidental observations. In many  
56 ways our purpose in this paper is to pull together many relevant incidental observations to bring  
57 into focus a coherent message that they seem to convey.

58

## 59 **Experimental**

60 All measurements of DMSP mentioned in this paper were carried out by alkaline  
61 hydrolysis of tissue,<sup>[12]</sup> followed by quantification of the produced DMS using gas  
62 chromatography. In our own research, each tissue subsample was placed in 25 mL of KOH  
63 solution (1 N or 2 N) in a glass vial sealed with a teflon-coated butyl rubber septum (Regis).  
64 After incubation for ca. 24 h, headspace gas was assayed for DMS by sulfur-specific gas  
65 chromatography employing a Chromosil 330 (Supelco) column at 54°C and Sievers 350A sulfur  
66 chemiluminescence detector. Standards were prepared using reagent DMS (Fluka) in background  
67 solutions that matched unknowns. We have previously reported evidence that the presence of  
68 animal tissue constituents does not affect measurement calibration.<sup>[9,13]</sup>

69 For our experiments on blue mussels (*Mytilus edulis*), the mussels were collected from

70 Vineyard Sound, Massachusetts, or an estuary near Sandwich, Massachusetts. All mussels in a  
71 given experiment were collected at the same place and time, and all were 6-8 cm long. To  
72 standardize mussel size, we first excluded individuals outside that size range, then chose subjects  
73 at random.

74         The laboratory experiments we report here consisted of three studies – termed the *10-day*,  
75 *2-week*, and *5-week Depuration Studies* – in which we deprived mussels of environmental  
76 sources of DMSP for a period (i.e., subjected them to depuration as discussed in the Results and  
77 Discussion), then fed measured amounts of DMSP to a subset of individuals, and then – 24 h  
78 after feeding – measured tissue accumulation in the fed and unfed mussels. In the *10-day*  
79 *Depuration Study*, we used relatively informal methods of depriving the mussels of  
80 environmental DMSP during the initial deprivation step. We simply withheld food and kept  
81 them in a sea table with routine, filtered, flowing seawater [ $0.3 \text{ nmol DMS(P) L}^{-1}$ ]. In the *2-* and  
82 *5-week Depuration Studies*, we used more-strict methods of depriving the mussels of DMSP  
83 during the initial deprivation step. Besides withholding food, we filtered all the water with  
84 which they came in contact through Gelman A/E glass fiber filters (nominal pore size  $1 \mu\text{m}$ ) to  
85 remove native DMSP-containing particulates (e.g., algal cells). Moreover, we housed the  
86 mussels throughout the deprivation period in groups of 5-6 individuals, each group in a separate  
87 3.8-L glass jar containing 2 L of filtered, aerated seawater. This seawater was changed only  
88 once each 24 h. With this procedure, the greatest amount of DMSP the mussels could obtain  
89 from their environment in 24 h was the DMSP available from 2 L of seawater that had passed  
90 through a Gelman A/E glass fiber filter.

91         To feed the mussels at the end of the deprivation step, we provided measured quantities  
92 of the DMSP-containing alga *Tetraselmis*, strain UW474, which is referable to *T. chunii* or *T.*

93 *suecica* (R. A. Lewin, pers. comm.). Average DMSP content at the stage of use was 27-42 fmol  
94 cell<sup>-1</sup>.

95 For analysis of tissue DMS(P) in mussels, each mussel usually was dissected into two  
96 parts: (1) the dark-colored digestive gland (consisting of the stomach, digestive diverticula, and  
97 associated tissues), hereafter called the *GI tissue* (gastrointestinal tissue); and (2) the rest of the  
98 body (including mantle, gills, nephridia, and adductor muscles), hereafter called the *Body tissue*.  
99 The GI tissue was so soft that we could subsample it with scissors; we minced it into small  
100 pieces, then mixed the pieces before taking a subsample. The Body tissue had to be processed  
101 differently because of the toughness of some of the body parts included. It was frozen in liquid  
102 nitrogen, then powdered with mortar and pestle while being kept frozen by additions of liquid  
103 nitrogen. The powder was stirred to create a homogeneous mix and subsampled. On occasion,  
104 we analyzed all the living tissue as a whole. In these cases, the entire body was frozen and  
105 powdered.

106 In the *10-day Depuration Study*, we deprived 20 mussels of environmental DMSP for 10  
107 days. We then assigned the mussels at random to 4 groups of 5 individuals, each group housed  
108 in its own 3.8-L glass jar. We fed 3 groups a measured quantity of DMSP (*Tetraselmis*, 3.8  
109  $\mu\text{mol DMSP group}^{-1}$ ), whereas one group continued to receive no food. After 24 h, each animal  
110 was subdivided into Body and GI tissue and analyzed.

111 In the *2-week Depuration Study*, we used 39 mussels. At random, we assigned 9 to be  
112 analyzed prior to environmental DMSP deprivation, and we subjected the other 30 to 2 weeks of  
113 environmental DMSP deprivation. In this case, the animals subjected to DMSP deprivation lived  
114 in groups of 5, each group in a separate 3.8-L jar, from the beginning of the deprivation period,  
115 as described already. At the end, 3 of these groups selected at random (termed Fed groups) were

116 fed *Tetraselmis* containing 3.7  $\mu\text{mol DMSP group}^{-1}$ , whereas the other 3 groups (termed Unfed  
117 groups) were not.

118 In the 5-week *Depuration Study*, we used larger numbers of mussels, subjected them to a  
119 longer depuration period, and then fed with a larger dose of DMSP. The mussels were collected  
120 in the wild, from a single large clump, just 1 week before the start of environmental DMSP  
121 deprivation. Because of the long period of environmental DMSP deprivation, we fed these  
122 mussels every other day during the deprivation period with a unialgal culture of *Dunaliella*  
123 (DUN) having no detectable DMSP. The study began with 58 mussels, 10 of which – chosen at  
124 random – were analyzed prior to environmental DMSP deprivation and 48 of which were  
125 assigned at the start, in groups of 6, to 8 glass jars at random. Six mussels were included in each  
126 group to guard against unplanned deaths. However, no animals died, and all groups were  
127 reduced to 5 animals near the end by removing a randomly selected individual. After 5 weeks of  
128 being deprived of environmental DMSP, 4 of the groups, selected at random, were fed  
129 *Tetraselmis* containing 5.0  $\mu\text{mol DMSP group}^{-1}$ , whereas the other 4 groups were not fed  
130 *Tetraselmis*.

131 In addition to the laboratory experiments, we carried out several descriptive field studies  
132 of *M. edulis* and ribbed mussels (*Geukensia demissa*). In these studies, we collected animals  
133 from their natural habitats (an estuary near Sandwich, Massachusetts, for *M. edulis*; Great  
134 Sippewissett Marsh, Falmouth, Massachusetts, for *G. demissa*) and, immediately after collection,  
135 analyzed their tissues by the methods already described. The specific goals of these field  
136 collections, and collection details, are presented along with the results in the Results and  
137 Discussion.

138 Statistical analyses were carried out in IBM SPSS Statistics, version 19. Normality

139 testing followed Park.<sup>[14]</sup> Specifically, we decided *a priori* to use the Shapiro-Wilks *W* statistic  
140 for reaching statistical decisions regarding the null hypothesis of a normal distribution. We also  
141 decided *a priori* to examine Q-Q plots.

142 For fitting an exponential model to data from the literature, coordinates of data points  
143 were read from the published graph. The dependent variable was then expressed as the natural  
144 logarithm, whereas the independent variable (time) was expressed in rectilinear coordinates. A  
145 line was fitted by linear regression, and the equation for the line was converted to exponential  
146 form.

147

## 148 **Results and Discussion**

### 149 *Depuration studies on blue mussels (Mytilus edulis)*

150 Depuration refers to the gradual decline of tissue DMS(P) when an animal is placed  
151 where it cannot further ingest DMS(P) or otherwise acquire DMS(P) from its environment.  
152 Depuration studies provide a means to examine tissue retention of DMS(P) because depuration  
153 and retention are inversely related (e.g., a low rate of depuration signifies high retention).  
154 Molluscs do not always lose DMS(P) when subjected to depuration conditions [i.e., a DMS(P)-  
155 free environment], meaning that depuration *per se* and depuration conditions sometimes need to  
156 be distinguished.

157 We first became aware of peculiarities in mollusc DMS(P) accumulation and retention  
158 when we attempted to complete a mass balance experiment on blue mussels, *Mytilus edulis*.<sup>[13]</sup>  
159 Our goal was to track the fate of ingested DMSP during the first 24 h following ingestion. One  
160 part of that research was the *10-day Depuration Study* (see Experimental), which was included  
161 because – after we fed the mussels the DMSP-containing phytoplankton (*Tetraselmis*) – we

162 needed to quantify the portion of the fed DMSP that they accumulated in their tissues and  
163 retained. To this end we employed an experimental design that not only seemed obvious and  
164 logical, but that also was identical to the design that we had used successfully to measure  
165 DMS(P) accumulation in fish.<sup>[9]</sup> We first subjected four groups of mussels (5 animals per  
166 group) to 10 days of depuration to lower the background concentration of DMS(P) in their  
167 tissues. Then we fed a measured amount of DMSP (3.8  $\mu\text{mol}$ ) to each of three groups, and after  
168 24 h we measured the amount of tissue DMS(P) in the Body and GI tissues of all individuals in  
169 all groups. We knew from contemporaneous measurements that in the Fed groups, the mussels  
170 rapidly removed *Tetraselmis* cells from the water when they were fed, and after the cells were  
171 removed, only 3% of the fed DMSP appeared in the environment in the form of DMSP or DMS  
172 during the 24 h following feeding.<sup>[13]</sup> Thus, we expected to find nearly all the fed DMSP  
173 accumulated in the tissues of the mussels.

174         However, the results did not substantiate tissue accumulation. Regardless of how one  
175 scrutinizes the data (Fig. 1), one cannot develop confidence that the results demonstrate  
176 accumulation in the tissues of the mussels. Consider, for example, Fed groups I and II. No  
177 information exists on the proportions of ingested DMSP that would be expected to be in the GI  
178 tissue or Body 24 h following ingestion. At first sight, the data for Fed groups I and II, when  
179 compared with the data for the Unfed group, might suggest that all the fed DMSP had  
180 accumulated in the GI tissue of the fed mussels. However, in both Fed groups I and II, the  
181 mussels collectively contained 5.9  $\mu\text{mol}$  in their GI tissue – an amount 4.7  $\mu\text{mol}$  higher than seen  
182 collectively in the GI tissue of the Unfed group (1.2  $\mu\text{mol}$ ) – even though each Fed group had  
183 received just 3.8  $\mu\text{mol}$  of DMSP in the *Tetraselmis* fed. In other words, Fed groups I and II  
184 contained too much DMS(P), compared to the Unfed group, for the amounts in their GI tissue to



185 be accounted for by feeding. Moreover, in Fed group III, the mussels collectively contained 2.6  
186  $\mu\text{mol}$  in their GI tissue, which exceeded the amount in the Unfed group (1.2  $\mu\text{mol}$ ) by less than  
187 40% of the fed amount, leaving 60% of the fed amount unaccounted for. If we assume that the  
188 DMSP provided to Fed groups I-III might have been partly or wholly in the Body tissue of the  
189 mussels at the time of analysis, we confront several ambiguities in the data, most notably that the  
190 Body tissue of one mussel in Fed group I contained 21.3  $\mu\text{mol}$ , almost 6 times as much DMS(P)  
191 as was fed to the whole group.

192 Before going further, we note that the data are presented in Fig. 1 as total amounts of  
193 DMS(P) per *animal* to permit simple visual accounting of body amounts relative to the amount  
194 fed. We have also analyzed the data in terms of DMS(P) per *gram* of tissue, but the ambiguities  
195 of interpretation are just as great. Similarly, in the follow-up studies we next discuss,  
196 interpretation is not altered whether we express the results as DMS(P) per animal or per gram.

197 We will not go further into the challenges of interpreting the results of particular  
198 experiments. That is not our purpose in this report.

199 Instead, what we want to stress here are the unusual statistical distributions of tissue  
200 DMS(P) in mussels and their implications. These statistical distributions are of significance in  
201 themselves, not merely because they confound data interpretation.

202 One striking aspect of the statistical distributions is the frequent occurrence of individuals  
203 that – according to visual inspection or statistical analysis – are high-valued outliers. In Fig. 1 at  
204 least two of the four sets of Body data include outliers. The Body DMS(P) amount in one  
205 individual in Fed group I is 5.2-21 times greater than that in the other individuals in the group,  
206 and in Fed group II the Body amount of one stands out by a factor of 2.0-3.2. As already noted,  
207 we find the same patterns whether we analyze DMS(P) per animal or per gram. Another striking

208 aspect of the statistical distributions is that they are often not normal. Again, this is true  
209 regardless of how the data are expressed. For testing normality of the data in Fig. 1, we lumped  
210 the data for all three Fed groups (I-III;  $n = 15$ ) and expressed DMS(P) content as DMS(P) per  
211 gram. Neither the Body nor the GI data are normally distributed, according to the Shapiro-Wilks  
212  $W$  test ( $W = 0.714$  and  $0.614$  in Body and GI tissue,  $p < 0.001$  in both)<sup>[14]</sup>. Nor are they normally  
213 distributed according to visual assessment of the Q-Q plots.<sup>[14]</sup>

214 After obtaining the results in Fig. 1, we undertook two follow-up studies – the *2-week*  
215 and *5-week Depuration Studies* – in the hope that we could obtain less ambiguous results on  
216 tissue DMS(P) accumulation following DMSP feeding by using larger sample sizes and  
217 subjecting the mussels to more prolonged, meticulous depuration procedures prior to feeding. In  
218 the *2-week Depuration Study*, after the mussels were subjected to depuration, the Fed groups  
219 received  $3.7 \mu\text{mol DMSP group}^{-1}$ , as shown in Fig. 2, and after 24 h, all mussels in the Fed and  
220 Unfed groups were analyzed.

221 The results (Fig. 2) were no clearer than the results of the *10-day Depuration Study* (Fig.  
222 1). Moreover, as in Fig. 1, nonnormal statistical distributions with severe outliers were a  
223 problem in drawing conclusions. Note, for example, that the Body tissue in a single unfed  
224 mussel in Unfed group I (Fig. 2) contained about the same amount of DMS(P) as the collective  
225 Body tissue in all 5 mussels in Fed group V, and a single fed mussel in group IV contained  
226 almost 3 times as much DMS(P) as had been fed to the entire group.

227 In the *5-week Depuration Study*, after the mussels were subjected to depuration  
228 conditions, the Fed groups received *Tetraselmis* containing  $5.0 \mu\text{mol DMSP group}^{-1}$ , as shown in  
229 Fig. 3. After 24 h, all mussels in the Fed and Unfed groups were analyzed, although in one Unfed

230 group (IV) and one Fed group (VIII), we analyzed the whole body of each individual, rather than  
231 subdividing into Body and GI parts.

232 If anything, the results of the *5-week Depuration Study* (Fig. 3) were even more  
233 ambiguous than those of the 2-week study. Nonnormal statistical distributions with severe  
234 outliers were again a major factor. For example, among the mussels subjected to the depuration  
235 procedure (i.e., the Fed and Unfed groups), the four individuals with highest Body DMS(P) were  
236 in Unfed groups, as were the three with highest GI DMS(P).

237

### 238 *Comparative studies of the rate of depuration in molluscs and fish*

239 We are aware of only one study on molluscs in the published literature in which the  
240 gradual loss of tissue DMS(P) under depuration conditions was measured quantitatively, namely  
241 Smit et al.'s study of abalone (*Haliotis midae*).<sup>[15]</sup> We are also aware of only one such study on  
242 fish.<sup>[16]</sup> In both the study on abalone and that on fish, the animals were enriched in tissue  
243 DMS(P) prior to depuration by feeding with *Ulva* seaweeds. The individual abalones and fish  
244 studied were similar in body size (20-50 g live tissue weight).

245 In fish, the general assumption of people in the field, based on practical experience, is  
246 that individuals with high tissue levels of DMS(P) depurate rapidly when placed on a DMS(P)-  
247 free diet. Levasseur et al.<sup>[8]</sup> report, for example, that when free-living Western Atlantic cod  
248 populations become enriched with tissue DMS(P) to a commercially detrimental extent, the  
249 problem lasts only 2-3 weeks.

250 Iida et al.<sup>[16]</sup> quantitatively described depuration in carp (presumably *Cyprinus carpio*)  
251 and rainbow trout (*Onchorhynchus mykiss*). Based on their data, the half-time for loss of tissue  
252 DMS(P) during depuration in both species was 1.1-2.1 days (Table 1).

253 In dramatic contrast, the half-time for DMS(P) loss in abalones was 27 days (Table 1).  
254 Recognizing the exponential nature of depuration, for tissue DMS(P) to fall 100-fold, the  
255 abalones required 182 days, whereas the carp and trout required only 12 days on average.

256 Our studies on *M. edulis* already discussed, although they do not permit calculation of  
257 depuration rate constants, suggest that some individual blue mussels do not undergo any  
258 depuration at all when deprived of dietary DMSP for 2-5 weeks. For example, in our *5-week*  
259 *Depuration Study* (Fig. 3), tissue levels of DMS(P) in one of the Unfed groups (II) were  
260 indistinguishable from levels in the Start group that was not subjected to the depuration  
261 procedure. More to the point, in both the 2- and 5-week *Depuration Studies*, at the end of the  
262 depuration procedure certain unfed individuals had tissue DMS(P) levels that ranked with the  
263 highest we recorded in the studies (Fig. 2, Fig. 3).

264

#### 265 *Statistical distributions in animals not fed following exposure to depuration conditions*

266 One set of statistical distributions is of particular interest in our studies of *M. edulis*: the  
267 distributions in mussels exposed to depuration conditions for 2-5 weeks and not fed prior to  
268 analysis (i.e., mussels in the Unfed groups, Fig. 2 and Fig. 3). These mussels had no inputs of  
269 DMSP from the start of the depuration period until their tissues were analyzed at the end. They  
270 thus provide direct insight into DMSP retention unconfounded with DMSP replacement.

271 Looking first at the *5-week Depuration Study* (Fig. 3), the statistical distribution of  
272 DMS(P) per unit tissue mass in the Unfed groups of mussels (all groups pooled) was highly  
273 nonnormal. We acquired data on DMS(P) per gram in the Body and GI tissues of 15 mussels  
274 (Unfed groups I-III, Fig. 3). In both tissues, the Shapiro-Wilks  $W$  statistic ( $n = 15$ ) and Q-Q plot  
275 point strongly to nonnormality (for Body,  $W = 0.775$ ,  $p < 0.002$ ; for GI tissue,  $W = 0.673$ ,  $p <$

276 0.0002). For those 15 mussels, we can also calculate the total DMS(P) per gram by combining  
277 the Body and GI results, providing data that can be lumped with the data for 5 additional mussels  
278 (Unfed group IV, Fig. 3) in which we directly measured total DMS(P). Total DMS(P) per gram  
279 in all 20 unfed mussels was dramatically nonnormal according to both the Shapiro-Wilks statistic  
280 ( $W = 0.685$ ,  $p < 0.00003$ ) and the Q-Q plot.

281         These nonnormal statistical distributions indicate that the DMS(P) metabolism of the  
282 unfed mussels subjected to the 5-week depuration period was not homogeneous. Instead the  
283 nonnormal statistical distributions suggest that there were physiological discontinuities among  
284 those mussels, meaning that – as we explain in this paragraph – there were divergent subsets of  
285 mussels. Visual inspection of particular data in Fig. 3 reinforces this conclusion. In the Start  
286 group ( $n = 10$ ), the lowest Body DMS(P) level was  $1.2 \mu\text{mol}$ . In Unfed groups I-III, the Body  
287 level was lower than that in 7 out of the 15 animals, suggesting that many mussels eliminated  
288 tissue DMS(P) when denied DMSP inputs for 5 weeks. By contrast, 5 mussels out of the total of  
289 15 in Unfed groups I-III finished the depuration period with Body DMS(P) levels as high as the  
290 levels seen in the upper 50<sup>th</sup> percentile of the Start group – suggesting that some mussels  
291 underwent little or no DMS(P) elimination when subjected to depuration conditions. In brief,  
292 there were two divergent subsets of mussels, one of which lost tissue DMS(P) during the 5  
293 weeks of exposure to depuration conditions, but the other of which seemed not to depurate much.  
294 Admittedly, these conclusions are conjectural. The nonnormal distribution itself interferes with  
295 orderly reasoning about the physiological significance of the data.

296         Looking now at the results of the *2-week Depuration Study* (Fig. 2), the statistical  
297 distribution of mass-specific DMS(P) concentration in the Unfed groups (considered  
298 collectively;  $n = 15$ ) was also nonnormal. Total DMS(P) per gram (calculated from the Body

299 and GI data) was nonnormal according to both the Shapiro-Wilks statistic ( $W = 0.88$ ,  $p < 0.05$ )  
300 and the Q-Q plot. DMS(P) per gram in the GI issue was nonnormal ( $W = 0.797$ ,  $p < 0.004$ ), and  
301 that in the Body tissue was only marginally normal ( $W = 0.884$ ,  $p = 0.05$ ).

302

303 *Statistical distributions in blue mussels (Mytilus edulis) and ribbed mussels (Geukensia demissa)*  
304 *in a single clump in the wild*

305 We have been impressed that there is typically a very large range of variation in the  
306 DMS(P) concentration per gram in individual mussels living in a single clump in the wild. The  
307 Start mussels in the *5-week Depuration Study* (Fig. 3) reflect this phenomenon, although they are  
308 not perfect examples because they had been in captivity for 1 week before they were analyzed,  
309 following collection in the wild. The Start individual with the highest Body DMS(P) content in  
310 Fig. 3 also had the highest mass-specific concentration:  $2.1 \mu\text{mol g}^{-1}$ . The Start individual with  
311 the lowest content in Fig. 3 had the lowest concentration:  $0.15 \mu\text{mol g}^{-1}$ . These two like-size  
312 mussels from a single clump therefore differed 14-fold in their concentration of DMS(P) per  
313 gram of living tissue.

314 To look directly at variation within clumps of *M. edulis* in the wild, we carried out field  
315 studies in which we collected and immediately analyzed four sets of *M. edulis* from a single  
316 marsh during each of four months in spring and summer. All animals each month ( $n = 15$ ) came  
317 from a single clump and were chosen at random from the mussels in the clump that were 6-8 cm  
318 long. For each animal, we analyzed all the tissue together and expressed results as DMS(P) per  
319 gram (Fig. 4; Seasons). In both July and August, the most concentrated mussel was 11 times  
320 richer in DMS(P) than the least concentrated. In April and May this ratio was lower but large, 6.4  
321 – 7.2. The statistical distributions in two months were nonnormal: May ( $W = 0.733$ ,  $p < 0.001$ )

322 and August ( $W = 0.855$ ,  $p < 0.05$ ). The distributions in April and July, on the other hand, were  
323 normal ( $W = 0.92 - 0.95$ ,  $p > 0.05$ ).

324         Mussels within a single clump in the wild would appear to feed in a relatively stereotyped  
325 way, being suspension feeders that primarily collect phytoplankton from ambient water they  
326 pump through their mantle cavities. One would imagine that the ambient water bathing two  
327 mussels of a single clump would be quite similar, especially when averaged over weeks or  
328 months of time. How can it be, then, that one mussel in a clump in the wild can have an order-  
329 of-magnitude more DMS(P) per gram than a near neighbor?

330         As part of our field work on *M. edulis*, we carried out a small study in which we  
331 categorized the mussels in a single clump as being in the interior or periphery of the clump. For  
332 statistical purposes, mussels at the two locations were paired *a priori* based on similar shell size.  
333 We collected two pairs from each of three clumps (during August of a different year than the  
334 Seasons collection) and measured total DMS(P) per gram (Fig. 4, Location in clump), as well as  
335 concentrations in the Body and GI tissues. We analyzed the results with both a nonparametric  
336 test (related-samples Wilcoxon signed rank) and a parametric test (paired *t*). In all cases (total,  
337 Body, and GI tissue), we obtained strong statistical evidence of no difference between the  
338 interior and peripheral mussels (paired *t*-test:  $p > 0.5$ ; Wilcoxon test,  $p > 0.5$ ).

339         We also examined whether the statistical distribution of DMS(P) per gram in *M. edulis* of  
340 a single clump is correlated with the elevation of the substrate to which the mussels were  
341 attached in an estuary with a sloping substrate. We set out four evenly spaced transects at a right  
342 angle to the axis of substrate slope, the lowest transect being subtidal and the others intertidal,  
343 with the highest about 1 m higher than the lowest. We then randomly selected and promptly  
344 analyzed 5 mussels at each elevation (i.e., along each transect). Mean total DMS(P) per gram

345 (Fig. 4, Location in estuary) did not vary significantly from the lowest to highest elevation  
346 (Kruskal-Wallis test,  $p > 0.7$ ). Based on this result, we pooled the data ( $n = 20$ ) to test normality  
347 and found the distribution of DMS(P) per gram to be strongly nonnormal ( $W = 0.51$ ,  $p <$   
348  $0.00001$ ). Similarly, mean DMS(P) per gram in the Body and GI tissues did not vary among  
349 elevations ( $p > 0.4$ ), and the data were nonnormal ( $p < 0.0001$ ).

350 To explore whether other mussel species exhibit the same types of statistical  
351 distributions, we analyzed data on freshly collected ribbed mussels, *Geukensia demissa*,  
352 collected at two sites (named A and B) near open water in the Great Sippewissett Marsh,  
353 Falmouth, Massachusetts ( $n = 15$  at each location). At both sites (Fig. 5), the individual with  
354 highest total DMS(P) per gram was about 8 times more concentrated than its neighbor with the  
355 lowest level. Moreover, total DMS(P) per gram was nonnormally distributed at both sites ( $W =$   
356  $0.78$  for site A,  $0.82$  for site B;  $p < 0.01$  for both). DMS(P) per gram in Body tissue was also  
357 nonnormal ( $p < 0.01$  for both sites), as was that in GI tissue ( $p < 0.0001$  for A,  $p < 0.01$  for B).

358 Of course, the concentration of DMS(P) in a mussel's tissue at a given time depends on  
359 the animal's preceding rates of gain and loss. One mussel could accumulate an order-of-  
360 magnitude higher concentration of DMS(P) than another while the two consume similar foods by  
361 assimilating dietary DMS(P) more completely. It could also do so by retaining assimilated  
362 DMS(P) more tightly. Differences in retention seem to us to be the more likely explanation for  
363 the high variation among neighbors within mussel clumps. One reason we say this is that our  
364 two efforts at finding correlations with feeding location (Fig. 4) indicated that it is not a factor.

365

366 *The highest tissue accumulations of DMS(P) in animals occur in molluscs*



367 To our knowledge, the animals that accumulate tissue DMS(P) to the highest mass-  
368 specific levels are molluscs. In wild-collected tridacnid clams *Tridacna crocea*, *T. maxima*, and  
369 *T. squamosa*, average concentrations of DMS(P) in the gill and byssal mantle tissues are 30-43  
370  $\mu\text{mol g}^{-1}$ .<sup>[10,11]</sup> These two tissues are separate in the body from the siphonal mantle, where the  
371 algal symbionts of the clams live. The tissues thus probably accumulate DMS(P) that is  
372 principally brought to them by blood flow.

373 The abalone *Haliotis midae* does not have algal symbionts. Nonetheless, it accumulates  
374 DMS(P) in its muscle tissue to concentrations averaging  $35 \mu\text{mol g}^{-1}$  when fed a diet rich in the  
375 seaweed *Ulva* in an aquaculture setting.<sup>[15]</sup>

376 These concentrations in wild *Tridacna* and aquacultured *Haliotis* exceed by  
377 approximately an order of magnitude the highest DMS(P) concentrations reported in other  
378 animals. Putting the concentrations in perspective is difficult, however, because unconfounded  
379 direct comparisons with other animals have not been carried out. Based on an earlier paper of  
380 ours,<sup>[10]</sup> DMS(P) concentrations higher than  $3\text{-}4 \mu\text{mol g}^{-1}$  are almost never observed in wild-  
381 collected animals of any kind other than *Tridacna* clams. The highest values in aquacultured fish  
382 fed DMSP supplements are  $4\text{-}8 \mu\text{mol g}^{-1}$ ,<sup>[17]</sup> far lower than in aquacultured abalones, *Haliotis*.<sup>[15]</sup>

383 As noted in the previous section, tissue concentration depends dynamically on the  
384 interplay of inputs and retention. Distinctively tight retention, as we are arguing is common in  
385 molluscs, would contribute to exceptional tissue concentrations in *Tridacna* and *Haliotis*.

386

### 387 *Pteropods as DMSP vectors*

388 Pteropods (planktonic molluscs) are well documented to be principal vectors for  
389 commercially detrimental accumulations of DMS(P) in fish such as chum salmon

390 (*Oncorhynchus keta*) and cod (*Gadus morhua*).<sup>[8,17,18]</sup> The pteropods feed directly or indirectly  
391 on DMSP-producing phytoplankton, and the fish obtain DMSP when they feed on the pteropods.  
392 Certainly much of the DMSP fish receive from eating pteropods comes from the pteropod  
393 stomach contents. It is therefore unfortunate that no studies seem to have been done to  
394 distinguish DMSP in the stomach contents from that assimilated into the pteropod tissues.  
395 Reasoning from the retentiveness for DMS(P) seen in some other molluscs, possibly pteropods  
396 accumulate and retain DMS(P) in their tissues to an exceptional extent, compared with other  
397 types of zooplankton of similar tiny body size. Such accumulation and retention would help  
398 explain their particular importance in passing DMSP up the food chain to fish.

399

#### 400 *Conclusions*

401 Sometimes the obstacles in research are the discovery. The obstacles in our laboratory  
402 experiments on blue mussels (*M. edulis*) compelled us to look at the data in terms of ranges and  
403 statistical distributions, rather than just averages. In doing so we realized that many individual  
404 *M. edulis* have relatively high accumulations of DMS(P) in their tissues and seem to retain  
405 DMS(P) exceptionally tightly. This observation led us to recognize other evidence of high  
406 accumulation and tight retention in the meager literature on DMS(P) in molluscs.

407 A particularly intriguing discovery is that all *M. edulis* are not alike. Order-of-magnitude  
408 ranges in DMS(P) accumulation occur routinely in close neighbors within groups of *M. edulis*  
409 living in the wild.. In addition, nonnormality is common, suggesting discontinuities in the ways  
410 neighbors accumulate and retain DMS(P).

411 For a full understanding of the biogeochemistry of DMSP and DMS in many ecosystems,  
412 processing by molluscs will need to be far better understood than it is today because molluscs

413 can be so abundant in local ecosystems that they are in a position to be major players. In this  
414 context it is well to recall that when oysters (*Crassostrea virginica*) were still at their primordial  
415 abundance 2-3 centuries ago, they were truly keystone animals in coastal communities,  
416 processing the entire water volume of large estuaries every few days.<sup>[19]</sup> In future experimental  
417 designs to advance biogeochemical knowledge of the roles of molluscs, the unusual  
418 accumulation and retention properties that we have highlighted will be essential to recognize.

419

420

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### Table legend

**Table 1.** Exponential models of loss of DMSP (depuration) from muscle tissue after animals were denied DMSP in their diet. The equation for abalone is from the original paper,<sup>[15]</sup> using an exponent that is the average of two slightly different values reported there. Equations for fish are calculated from the original data<sup>[16]</sup> over the time period from the time of highest DMSP concentration to day 13. In the original research on fish<sup>[16]</sup>, two studies were done on each fish species, one study in which the fish were fed 1% *Ulva* prior to depuration and another in which they were fed 5% *Ulva*. This explains why we present two sets of results for each species. Half-times for DMSP loss are calculated from exponents.

**Figure legends**

**Fig. 1.** Results of the *10-day Depuration Study* on *Mytilus edulis*. Each symbol refers to one individual. Closed and open symbols show total DMS(P) content ( $\mu\text{mole}$ ) in Body and GI tissue, respectively. Arrow on ordinate shows the amount of DMSP fed to each Fed group ( $3.77 \mu\text{mol group}^{-1}$ ) 24 h before the mussels were analyzed.

**Fig. 2.** Results of the *2-week Depuration Study* on *Mytilus edulis*. Each symbol refers to one individual. Closed and open symbols show total DMS(P) content ( $\mu\text{mole}$ ) in Body and GI tissue, respectively. Animals in the Start group were analyzed at the start of the study, prior to exposure to depuration conditions. Those in the Fed and Unfed groups were analyzed at the end, after 2 weeks of exposure to depuration conditions. Arrow on ordinate shows the amount of DMSP fed to each Fed group ( $3.71 \mu\text{mol group}^{-1}$ ) 24 h before the end.

**Fig. 3.** Results of the *5-week Depuration Study* on *Mytilus edulis*. Each symbol refers to one individual. Closed and open symbols show total DMS(P) content ( $\mu\text{mole}$ ) in Body and GI tissue, respectively. Squares show total DMS(P) content ( $\mu\text{mole}$ ) in the Body and GI tissues combined. Animals in the Start group were analyzed at the start of the study, prior to exposure to depuration conditions. Those in the Fed and Unfed groups were analyzed at the end, after 5 weeks of exposure to depuration conditions. Arrow on ordinate shows the amount of DMSP fed to each Fed group ( $4.95 \mu\text{mol group}^{-1}$ ) 24 h before the end of the study.

**Fig. 4.** Total DMS(P) per gram of living tissue in *Mytilus edulis* immediately after collection in the wild. Six independent collections are included: four “Seasons” collections carried out in each of four months of one year ( $n = 15$  per month); a “Location in clump” collection in which mussels in the interior and periphery of clumps were compared; and a “Location in estuary” collection, in which mussels on a sloping substrate were compared as a function of substrate elevation. The latter two collections were conducted in August three years after the August “Seasons” collection. Each symbol refers to one individual. The symbol marked with an asterisk should be plotted at  $7.1 \mu\text{mol g}^{-1}$ .

**Fig. 5.** Total DMS(P) per gram of living tissue in ribbed mussels (*G. demissa*) immediately after collection in the wild. Data are for two sites (A and B) on the banks of low-order tidal creeks within a *Spartina alterniflora* salt marsh (Great Sippewissett Marsh, Falmouth, MA). At each site, 15 mussels were collected at random. These are unpublished data from Bradley A. White.