Exceptional accumulation and retention of dimethylsulfoniopropionate by molluscs

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Introduction

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

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

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

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

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

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

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

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

Experimental

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

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

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

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

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

93 *suecica* (R. A. Lewin, pers. comm.). Average DMSP content at the stage of use was 27-42 fmol
94 $cell^{-1}$.

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 μmol 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

fed *Tetraselmis* containing 3.7 μmol DMSP group^{-1}, whereas the other 3 groups (termed Unfed groups) were not.

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

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

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

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

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

Results and Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Comparative studies of the rate of depuration in molluscs and fish

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

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

Iida et al.[16] quantitatively described depuration in carp (presumably *Cyprinus carpio*) and rainbow trout (*Onchorhynchus mykiss*). Based on their data, the half-time for loss of tissue 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, $W = 0.673$, $p <$

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The abalone *Haliotis midae* does not have algal symbionts. Nonetheless, it accumulates DMS(P) in its muscle tissue to concentrations averaging 35 μmol g^{-1} when fed a diet rich in the seaweed *Ulva* in an aquaculture setting.[15]

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

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

Pteropods as DMSP vectors

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

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

Conclusions

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

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

For a full understanding of the biogeochemistry of DMSP and DMS in many ecosystems, 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.[19] 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,[15] using an exponent that is the average of two slightly different values reported there. Equations for fish are calculated from the original data[16] over the time period from the time of highest DMSP concentration to day 13. In the original research on fish[16], 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 (μmole) in Body and GI tissue, respectively. Arrow on ordinate shows the amount of DMSP fed to each Fed group (3.77 μmol 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 (μmole) 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 μmol 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 (μmole) in Body and GI tissue, respectively. Squares show total DMS(P) content (μmole) 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 μmol 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 μmol 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.