

## Dimethylsulfoniopropionate in Giant Clams (Tridacnidae)

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**Abstract.** The tridacnid clams maintain symbiotic associations with certain dinoflagellates (termed zooxanthellae). Tridacnids are thus candidates to have high tissue concentrations of dimethylsulfoniopropionate (DMSP), a tertiary sulfonium compound that is not synthesized by animals but is commonly produced by dinoflagellates. This study establishes that DMSP is about an order of magnitude more concentrated in the light-exposed and shaded mantle and gills of *Tridacna maxima* and *T. squamosa* than in any other known animal tissues. The DMSP concentration in the light-exposed, siphonal mantle—the location of most zooxanthellae—is an inverse function of body size, paralleling an inverse relation between apparent density of zooxanthellae (measured as pheophytin concentration) and body size. The shaded mantle and gills are high in DMSP despite having low densities of zooxanthellae, indicating that high DMSP concentrations occur in molluscan tissue, not just in algal cells. DMSP is almost an order of magnitude less concentrated in the adductor muscle than in other tissues. The high DMSP concentrations found in tridacnids, by providing abundant substrate for formation of volatile dimethylsulfide, probably explain the peculiar tendency of tridacnids to rapidly develop offensive odors and tastes after death: a serious problem for their exploitation as food. Tridacnids are the one group of animals in which DMSP concentrations are high enough in some tissues to be in the range capable of perturbing enzyme function at high physiological temperatures. Thus, tridacnids may require enzyme forms adapted to DMSP.

### Introduction

Some of the most interesting marine animals are those that maintain symbiotic associations with dinoflagellates. The symbiotic dinoflagellates are known as zooxanthellae. Included are the reef-building scleractinian corals, many alcyonarians, all of the about eight species of giant clams of the family Tridacnidae, and a few other bivalves (Maruyama *et al.*, 1998). Dinoflagellates, as a group, are noteworthy for synthesizing relatively large quantities of dimethylsulfoniopropionate (DMSP), a nonvolatile tertiary sulfonium compound that is the precursor of volatile dimethylsulfide (DMS) (Keller *et al.*, 1989a,b). DMSP is not synthesized endogenously by animals. However, the widespread synthesis of DMSP by dinoflagellates provides reason to predict accumulations of DMSP and DMS within the tissues of zooxanthellate animals. This prediction has been assessed heretofore only in reef-building scleractinians. The presence of the reduced-sulfur compounds in reef-building corals was confirmed initially by the observation of DMS release from damaged reefs (Andreae *et al.*, 1983). Later, two of us quantified DMSP and DMS in the tissues of healthy corals and in free-living dinoflagellates isolated from corals (Hill *et al.*, 1995b).

In giant clams, the zooxanthellae, which currently are assigned to two of the major subdivisions of *Symbiodinium* (Rowan, 1998), occur primarily in the siphonal mantle tissue (Norton *et al.*, 1992). This expansive tissue faces upward when the clams are in their natural orientation and is presented to the sun as a light antenna. The part of the mantle that is positioned near the downward-facing byssal opening and hinge, shaded from the sun, contains relatively few zooxanthellae (documented in this study). Similarly, zooxanthellae are sparse or absent from the adductor muscle, gills, and other tissues besides the siphonal mantle.

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**Abbreviations:** DMS, dimethylsulfide; DMSP, dimethylsulfoniopropionate.

Recent molecular evidence confirms that the giant clams are a monophyletic group (Maruyama *et al.*, 1998). This paper, based on two species, is the first to look for or quantify DMSP and DMS in the group. It is also the first to examine DMSP and DMS in zooxanthellate animals besides reef-building scleractinians.

DMSP and DMS are of current interest for several reasons. The most prominent is that atmospheric DMS originating from marine organisms affects cloud cover and climate over the oceans (Shaw, 1983; Charlson *et al.*, 1987; Falkowski *et al.*, 1992; Andreae and Crutzen, 1997). Coral reefs are sufficiently extensive that DMS from corals could be of local climatic significance (Andreae *et al.*, 1983; Hill *et al.*, 1995b). However, DMS from giant clams seems unlikely to be climatically important except as a minor component of reef-community contributions, because the clams are insufficiently abundant, especially in modern times. On the other hand, DMSP and DMS in giant clams are likely important in two major ways.

First, DMS is well known to have critical effects on taste whenever it is present in organisms used for food (Motohiro, 1962; Ackman *et al.*, 1966; Lévassieur *et al.*, 1994). Giant clams have long been important sources of food and protein throughout much of the Indo-Pacific, so much so that many clam populations are decimated (Munro, 1989; Dalzell *et al.*, 1996). A problem for the indigenous and commercial exploitation of giant clams is that after death, the meat often promptly develops a strong, "unquestionably offensive" odor (Peavey and Riley, 1993, 1994), which is sometimes described as seaweed- or kelp-like (*e.g.*, Cowan, 1988). The cause has been unknown. Our experience with DMS and with the odors of the clams led us to postulate that the cause is DMS derived from the algal symbionts. If this hypothesis is confirmed, the stage will be set for a rational approach to a problem that seriously detracts from the value of the clams as sources of food in subsistence economies and as aquacultured species. Giant clams are attractive animals for aquaculture (Braley, 1988; Munro, 1989) in part because their symbionts enable them to get most of their energy for maintenance and growth from sunlight (Klumpp and Griffiths, 1994); they have been described as "the only phototrophic, and thus self-feeding, potential farm animals known to humankind" (Munro, 1989).

Second, recent research (Nishiguchi and Somero, 1992; Karsten *et al.*, 1996) has established that DMSP sometimes negatively perturbs enzyme function at high physiological temperatures. The enzyme-perturbing effects of DMSP have heretofore been considered relevant only to plants and algae, because no animals have been known to have DMSP concentrations sufficiently high to be influential. We hypothesized that the giant clams might have tissue DMSP concentrations high enough that they could potentially require biochemical adaptations to DMSP.

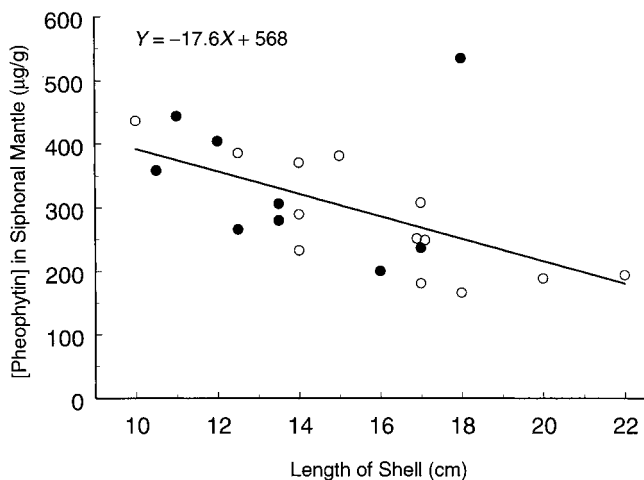
## Materials and Methods

*Tridacna maxima* and *T. squamosa* were collected near Pohnpei in the Federated States of Micronesia. Two collections were made: one of six specimens of *T. maxima* (11–14 cm shell length) and four of *T. squamosa* (10–14 cm) on 13 July 1995, on the northern barrier reef of the main island of Pohnpei; the second of three specimens of *T. maxima* (16–18 cm) and nine of *T. squamosa* (14–22 cm) on 17 August 1995, in the lagoon of Ant atoll, 27 km from the first location. Collected animals were taken by boat to Kolonia, Pohnpei, where they were promptly dissected (3–4 h after collection). Samples (1–2 g) were cut from four tissues of each clam: light-exposed, siphonal mantle; shaded mantle from near the byssal opening; adductor muscle; and gill (sometimes both right and left gill sets combined). Each sample of tissue was weighed and placed into 20 ml of HPLC-grade absolute methanol in a 37-ml glass vial sealed with a Teflon-faced butyl-rubber septum (Regis Technology) secured with a crimped aluminum ring.

Samples prepared in Pohnpei were shipped in light-tight containers to Woods Hole, Massachusetts, for assay. Shipments required 6–8 days to reach Woods Hole, and assays were completed 8–10 days after collection. Vials had been filled with methanol and weighed prior to shipment to Pohnpei, and they were reweighed on return to Woods Hole as a check for leakage (none occurred).

To measure DMSP and DMS, 1.0 ml of methanol was drawn by syringe from each tissue-sample vial and placed in 25 ml of 2 N KOH in a sealed vial. Incubation in cold base quantitatively converts DMSP to DMS (Dacey and Blough, 1987). Thus, after incubation (20 h, 2°C), DMSP (plus any DMS present in the initial samples) could be measured by assaying DMS. For assay, the vials of KOH were brought to room temperature (*ca.* 22°C), and DMS was measured in head-space samples by gas chromatography, using a Chromosil 330 (Supelco) column at 54°C for separation, Sievers 350B sulfur chemiluminescence detector, and Hewlett Packard 3390A integrator. Standards were prepared in 25 ml of 2 N KOH plus 1 ml methanol using reagent grade DMS (Fluka). All measures were duplicated.

Chlorophyll in each tissue sample was measured as an index of the density of zooxanthellae. In fact, because some chlorophyll could have degraded to pheophytin during sample preparation and shipment, and because our interest was not in chlorophyll itself but in an index of relative levels of zooxanthellae, we degraded all chlorophyll by acidification (3 mM HCl) and used the resulting total pheopigment levels as our index (Hill *et al.*, 1995b). Chlorophyll *a* and pheopigment *a* were measured using a calibrated Turner model 10 fluorometer and standards of chlorophyll *a* from spinach (Sigma) in methanol following procedures recommended by Holm-Hansen and Reimann (1978). Aliquots of methanol drawn by syringe from tissue-sample vials were diluted in



**Figure 1.** Pheophytin *a* per gram of siphonal mantle (wet weight) as a function of shell length in *Tridacna maxima* (filled symbols) and *T. squamosa* (open symbols). The line and equation are results of linear, least-squares regression, all data taken together. Two data points for 17-cm clams are shifted laterally for clarity.

absolute methanol to place concentrations on the linear parts of calibration curves.

In Pohnpei, tissue samples were prepared, weighed, and inserted in vials as solid blocks of tissue to minimize potential loss of DMS. To assure that extractions of DMSP, DMS, and chlorophyll from samples into methanol were complete, tissue samples were removed briefly from sample vials after completion of the measurements described above and minced with scissors into small pieces (*ca.* 1 mm greatest thickness) that fell back into the vials (the procedure required about 1 min per sample). After 24–48 h, all measurements were repeated. Chlorophyll concentrations were not altered by mincing, and concentrations of pooled DMSP and DMS (measured as earlier described) were altered little, if at all (possibly 3%–5% in the case of mantle samples). The mincing test demonstrated that extraction from whole tissue was complete or virtually complete, and all assays for a sample were averaged to obtain the results reported. A second check on our technique was to test whether the high tissue DMSP concentrations we encountered might be so high as to saturate the methanol. The highest concentration of DMSP in the methanol in any tissue-sample vial was 4.3 mM. Without attempting to determine the absolute solubility of DMSP in methanol, we ascertained that pure DMSP sufficient to make a solution three times as concentrated dissolved rapidly in methanol. Thus, saturation of the methanol in the sample vials did not occur.

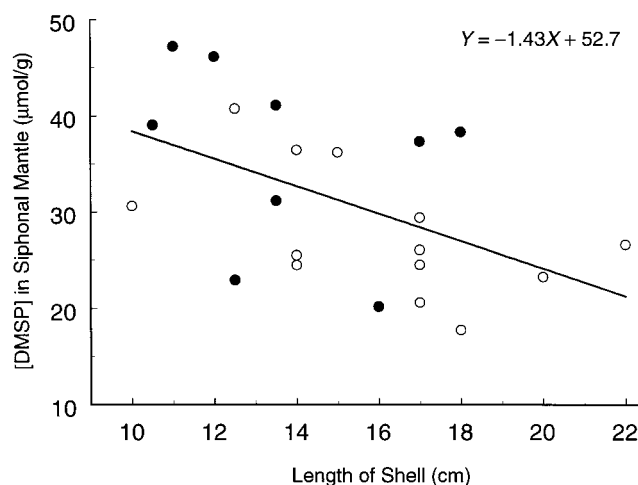
Total amounts of pooled DMSP and DMS (in micromoles) and of pheopigment *a* (in micrograms) in tissue samples were calculated from concentrations in sample-vial methanol by multiplying by the volume of methanol (20 ml), then expressed per unit wet-weight of tissue.

## Results

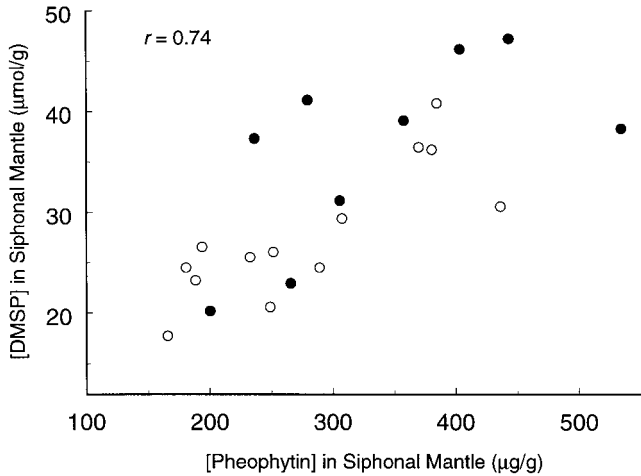
For simplicity of language, we express results in terms of DMSP, although we do not know what proportions of the DMS analyzed were initially in the form of DMS or DMSP. Most was probably DMSP (the nonvolatile form known to be the principal chemical species in algae; see also comparative data on molluscs presented later). Results from *Tridacna maxima* and *T. squamosa* were not statistically distinguishable and thus are generally pooled.

Pheophytin *a* per gram of siphonal (light-exposed) mantle was an inverse function of body size (analyzed by linear regression,  $P = 0.008$ ,  $r^2 = 0.30$ ), as shown in Figure 1. DMSP per gram of siphonal mantle was likewise an inverse function of body size ( $P = 0.015$ ,  $r^2 = 0.26$ ), as shown in Figure 2. The concentrations of DMSP and pheophytin in the siphonal mantle were strongly correlated ( $P < 0.001$ ), as shown in Figure 3. The ratio of DMSP concentration to pheophytin concentration in siphonal mantle was quite consistent, the mean and standard error being  $0.107 \pm 0.0048$  (range: 0.070–0.158)  $\mu\text{mol}/\mu\text{g}$ .

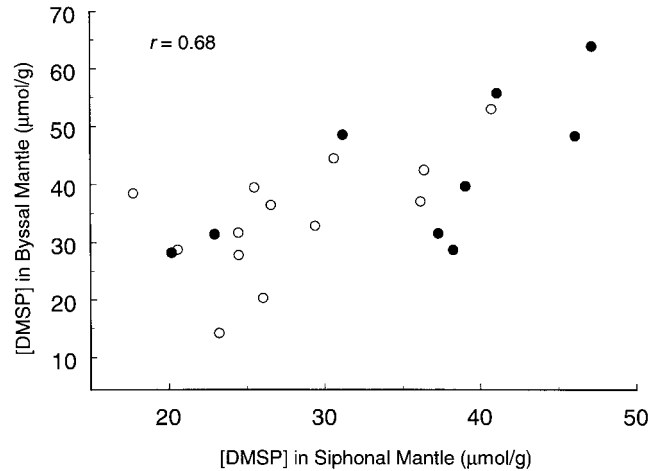
Pheophytin per gram did not show a systematic relation to body size in the byssal (shaded) mantle, adductor, or gill. Table 1 summarizes the pheophytin concentrations in these tissues. Comparison to Figure 1 shows that the concentrations were far lower than in the siphonal mantle. The simple mean concentration in the siphonal mantle, 303  $\mu\text{g}/\text{g}$ , is of uncertain utility because of the regular relation between siphonal-mantle concentration and body size, but it helps bring to light that pheophytin concentrations in the byssal mantle and gill were only about 7% and 3% as high as those in siphonal mantle. Concentrations of pheophytin in the adductor approached zero.



**Figure 2.** Concentration of dimethylsulfoniopropionate (DMSP) in siphonal mantle (wet weight) as a function of shell length in giant clams. The line and equation are results of linear, least-squares regression, all data taken together. Filled symbols, *Tridacna maxima*; open symbols, *T. squamosa*.



**Figure 3.** Correlation between concentrations of dimethylsulfoniopropionate (DMSP) and pheophytin *a* in siphonal mantle of giant clams. Filled symbols, *Tridacna maxima*; open symbols, *T. squamosa*. *r* = correlation coefficient.



**Figure 4.** Correlation between concentrations of dimethylsulfoniopropionate (DMSP) in byssal and siphonal mantle in giant clams. Filled symbols, *Tridacna maxima*; open symbols, *T. squamosa*. *r* = correlation coefficient.

As shown in Figure 4, the concentration of DMSP in the byssal mantle tissue was strongly correlated with ( $P < 0.001$ ) and similar to that in the siphonal mantle tissue, even though densities of zooxanthellae in the byssal tissue, as inferred from byssal pheophytin concentrations, were a small fraction of those in the siphonal tissue. Presumably because of the correlation between siphonal and byssal concentrations, the byssal DMSP concentration exhibited a regular relation to body size, similar to that in Figure 2 [linear regression:  $Y(\mu\text{mol/g}) = -2.46X(\text{cm}) + 74.4$ ;  $P = 0.001$ ]. Byssal DMSP concentration showed no correlation with byssal pheophytin concentration.

The DMSP concentrations in gill and adductor were

unrelated to body size and uncorrelated with pheophytin concentrations in the respective tissues. The DMSP concentration in gill was positively correlated with that in mantle ( $r = 0.61$ ,  $P < 0.01$  for siphonal mantle;  $r = 0.45$ ,  $P < 0.05$  for byssal mantle), but the DMSP concentration in adductor was not correlated with that in mantle. Table 2 presents DMSP concentrations in gill and adductor. For comparison, the means in siphonal and byssal mantle were 31.2 and 37.4  $\mu\text{mol/g}$  (see Fig. 4). Note that gill exhibits DMSP concentrations similar to those of mantle. In adductor, however, DMSP is almost an order of magnitude less concentrated than in mantle.

**Discussion**

DMSP in the mantle and gill tissues of *Tridacna maxima* and *T. squamosa* is far more concentrated than in any animal tissue heretofore known. Most comparative data in the literature represent pooled concentrations of DMSP and DMS (similar to the data we collected). In discussing the literature, we distinguish DMSP and DMS only if the orig-

**Table 1**

*Pheophytin a per gram of tissue of giant clams (wet weight, species combined) in the three tissues that showed no relation between concentration and body size*

Tissue	Pheophytin <i>a</i> ( $\mu\text{g/g}$ )	
	Mean	Range
Byssal mantle*	22.10	1.34–86.90
Gill	10.00	0.35–31.30
Adductor muscle	0.74	0.05–3.32

Data for siphonal mantle are omitted because they are presented elsewhere (Fig. 1) and because the mean is a possibly misleading statistic for a parameter that varies systematically with body size.

\* The four highest values for byssal mantle occurred in four of the smallest clams (two of each species), suggesting that the effort to keep tissue-sample size consistent might have led to the inclusion of other types of tissue in byssal-mantle samples of some small clams. If the four highest values are excluded, the mean and range for byssal mantle are 12.8 and 1.34–33.1  $\mu\text{g/g}$ .

**Table 2**

*Dimethylsulfoniopropionate (DMSP) per gram of tissue of giant clams (wet weight, species combined) in the two tissues that showed no relation between concentration and body size*

Tissue	DMSP ( $\mu\text{mol/g}$ )	
	Mean	Range
Gill	33.3	20.3–46.1
Adductor muscle	4.4	1.8–7.2

Data for mantle are omitted for reasons stated in note to Table 1.

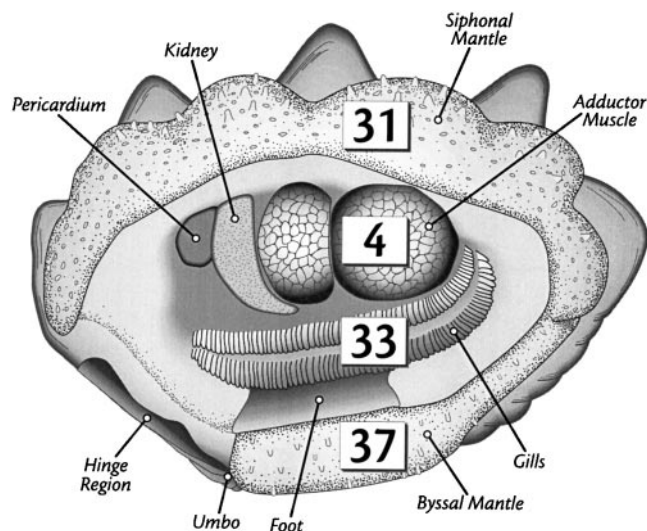
inal investigators did. We also exclude from consideration tissues (*e.g.*, stomach) that could contain unassimilated food. Two surveys of DMSP concentrations in molluscs have been carried out. Iida and Tokunaga (1986) measured both DMS and DMSP in 11 species of bivalves and 5 of gastropods from Japanese waters. An average of 8% of the total molar amount of the two compounds was DMS and 92% was DMSP in the mantle, gill, and adductor tissues of the bivalves. The sum of the two concentrations was usually less than 0.2  $\mu\text{mol/g}$ . The single highest sum was 0.9  $\mu\text{mol/g}$  in adductor muscle of oysters (*Crassostrea gigas*). Ackman and Hingley (1968) reported 0–1.8  $\mu\text{mol/g}$  in the tissues of 10 species of bivalves and gastropods from Canadian waters. Most values were toward the low end of the range; the highest concentrations were in adductor muscles of scallops (*Placopecten megallanicus*) and oysters (*C. virginica*). The highest concentrations observed in populations of mussels (*Mytilus edulis*) at Cape Cod, Massachusetts, were 2–4  $\mu\text{mol/g}$  (Hill *et al.*, 1995a). In wild-caught fish, muscle or liver concentrations of 0.2–1.0  $\mu\text{mol/g}$  (predominantly DMSP in fresh tissue) are high and commercially problematic because they cause off-flavors (see later) (Motohiro, 1962; Ackman *et al.*, 1967; Iida *et al.*, 1986; Dacey *et al.*, 1994). Even the highest tissue concentrations in fish fed DMSP supplements were only 4–8  $\mu\text{mol/g}$  (Ackman *et al.*, 1966). A survey of DMS and DMSP in several species of shrimp and krill indicated that tissue concentrations are very low in most species, although pooled concentrations (predominantly DMSP) as high as 3  $\mu\text{mol/g}$  are sometimes observed in muscle of *Euphausia superba* (Tokunaga *et al.*, 1977). In the context of these comparative data, the concentrations in the mantle and gill tissues of tridacnids, averaging 31–37  $\mu\text{mol/g}$ , are extraordinary. The only published animal data that are at all in the same range come from a single report on pteropods (Levasseur *et al.*, 1994) in which extremes of 30–40  $\mu\text{mol/g}$  were observed (calculated from published data on tissue dry weights assuming the animals to be 70% water). Such concentrations, however, are exceptional in the literature on pteropods; other reports are 0.2–3.7  $\mu\text{mol/g}$  (Motohiro, 1962; Ackman and Hingley, 1968) or lower (Ackman *et al.*, 1972). Furthermore, the pteropod data are for whole animals, including digestive-tract contents. In terms of documented evidence on tissues of animals collected in the wild, the concentrations in tridacnid mantle and gill are extreme: an order of magnitude higher than the highest concentrations observed in other bivalve or gastropod molluscs, fish, crustaceans, and other animals. Other than the tridacnids, the animals mentioned acquire DMSP strictly from ingested foods.

We hypothesize that the high concentrations in tridacnids are a consequence of the production of DMSP by their algal symbionts. Three pieces of evidence support this hypothesis. First, at least in *T. squamosa*, symbiont photosynthesis supplies over 90% of all organic carbon acquired by indi-

viduals of the body sizes we studied (Klumpp and Griffiths, 1994). Thus, although the clams ingest food to some extent and could acquire DMSP from their food, the known partitioning between phototrophic and heterotrophic nutrition makes phototrophic production of DMSP the more likely source of most DMSP, especially because symbiotic dinoflagellates are documented to produce DMSP (Hill *et al.*, 1995b). Second, the sheer magnitude of the DMSP concentrations in the tridacnids points to their algal symbionts as the source, because in all the many species of studied molluscs that lack algal symbionts, even the most extreme DMSP concentrations seen do not come close to the routine concentrations seen in the tridacnids. Third, there are several reports that the density of zooxanthellae in tridacnid tissues is an inverse function of body size (*e.g.*, Fisher *et al.*, 1985; Griffiths and Klumpp, 1996), and we found evidence of this same relation in siphonal mantle (Fig. 1). If the zooxanthellae are the primary source of DMSP and if the zooxanthellae become less dense in the siphonal mantle as individuals grow, one would predict that the DMSP concentration would be an inverse function of body size. This is what we found (Fig. 2). The DMSP and pheophytin concentrations in the siphonal mantle were well correlated (Fig. 3), and the ratio of the concentrations was consistent at  $0.107 \pm 0.0048$  (SE)  $\mu\text{mol}/\mu\text{g}$ .

If our hypothesis is correct that the zooxanthellae are the main source of DMSP in giant clams, then tissues other than the siphonal mantle must receive DMSP by internal transport from the siphonal mantle because, as our pheophytin data confirm, the zooxanthellae occur at high densities only in siphonal mantle (Table 1). Internal transport could occur by circulation of hemolymph or by transport in the zooxanthellal tubular system (Norton *et al.*, 1992). Molluscan tissues are well known to accumulate DMSP (Ackman and Hingley, 1968; Hill *et al.*, 1995a). The high concentrations of DMSP in siphonal mantle could possibly be explained by high concentrations in the algal cells only. However, the byssal mantle and gills are so low in algal cells that the high concentrations of DMSP there almost certainly demonstrate that the animal cells of giant clams can experience very high DMSP concentrations. The physiological basis for the dramatic difference in concentration between the adductor muscle and other tissues (Fig. 5) awaits study.

Whatever the cause of the high DMSP concentrations in tridacnids, the concentrations are likely to be important to the biology of the clams in two major ways. The first is taste. One of the principal conclusions of research on commercialization of giant clams is that their meat is exceptionally perishable because of the rapid development of a “particularly offensive and pervasive odor” (Peavey and Riley, 1994). Refrigeration (Peavey and Riley, 1994) or freezing in a domestic freezer (Peavey and Riley, 1993) does little to prevent this problem, even if the viscera have been removed. Comparing mantle and adductor meat, a disagree-



**Figure 5.** A giant clam in the natural orientation showing average pooled concentrations ( $\mu\text{mol/g}$ ) of dimethylsulfoniopropionate (DMSP) and dimethylsulfide (DMS) in the four tissues studied (drawing by Jayne Doucette).

able “seaweed-like” odor has been particularly associated with the mantle (Cowan, 1988), and the mantle is far less commercially valuable than the adductor (Braley, 1988; Tisdell and Tacconi, 1993). These are serious matters in many parts of the world where giant clams occur. The clams are sources of food and protein in subsistence economies, and a number of cash-poor governments are making substantial investments in the development of giant clam aquaculture (Lucas, 1994). Partly because of preservation problems, the mantle meat may actually have a negative monetary value in commercial aquaculture (Hambrey and Gervis, 1993).

We believe we have discovered the cause of the unattractive odor and taste, thereby setting the stage for a rational approach to improvement. Although DMS has not been mentioned previously as a likely component of tridacnid tissues, it is well recognized as an important taste constituent in other seafoods. DMS produced from dietary DMSP is a negative taste factor in fish. The DMS generated during certain sorts of processing of fish meat containing as little as  $0.1\text{--}1\ \mu\text{mol DMSP/g}$  can cause the meat to smell “like petroleum” or taste like turnip or radish (Motohiro, 1962; Ackman *et al.*, 1966) and force catches to be discarded. On the other hand, very low concentrations of DMS are part of the valued flavor of some clams and oysters (Ackman and Hingley, 1968; Brooke *et al.*, 1968; Iida and Tokunaga, 1986). Brooke *et al.* (1968), for example, found that about  $0.02\ \mu\text{mol/g}$  of DMS helps impart a desirable “clamlike” odor to *Mya arenaria*, whereas more than  $0.3\ \mu\text{mol/g}$  of DMS is excessive.

Our data reveal that the potential for DMS formation in tridacnid tissues, particularly mantle, is enormous. We as-

sume that in the fresh clams we studied, most of the sulfur we measured was in the form of DMSP (Iida and Tokunaga, 1986), which has unknown taste effects. After the death of the clam, DMSP is likely under many circumstances (Motohiro, 1962; Ackman *et al.*, 1966) to be broken down to DMS enzymatically (*e.g.*, by bacterial DMSP lyases; Ledyard *et al.*, 1993) or nonenzymatically (*e.g.*, Dancey and Blough, 1987). With over  $30\ \mu\text{mol/g}$  of DMSP present, the concentrations of DMS that could readily be formed are far in excess of ones known to make all other foods inedible. It is probably no accident that the methods devised by indigenous people to preserve tridacnid meat include acidic washes and drying (Munro, 1989; Hambrey and Gervis, 1993). Acid pHs inhibit the nonenzymatic breakdown of DMSP to DMS (Motohiro, 1962; Dacey and Blough, 1987), and because DMS is very volatile, drying would remove it from tissue. Looking to the future, it might be possible to inhibit DMS formation in a chemically specific manner or even to develop strains of zooxanthellae that produce little DMSP.

The second major way in which DMSP concentrations are potentially important to tridacnid biology is their biochemical significance. Recognizing that DMSP is employed by some organisms to help set the colligative properties of cellular solutions, Nishiguchi and Somero (1992) studied the effects of DMSP on cellular proteins. They found evidence of temperature dependence. Whereas DMSP exhibited stabilizing effects on proteins at low temperatures, it could perturb protein function at high physiological temperatures. In particular, Nishiguchi and Somero found that DMSP promotes the denaturation of glutamate dehydrogenase at  $37^\circ\text{C}$  in a concentration-dependent manner, with effects evident at the lowest concentration tested,  $100\ \text{mM}$  DMSP. Similarly, Karsten *et al.* (1996) observed a concentration-dependent suppression of activity of malate dehydrogenase at  $30^\circ\text{C}$ ; the suppression became evident at concentrations between  $19$  and  $75\ \text{mM}$  DMSP. Such effects of DMSP have been tacitly assumed to be relevant just to plants and algae because only plants and algae have hitherto been thought to have native DMSP concentrations high enough to be in the effective range. Our results make clear that alone among animals, tridacnids can have DMSP concentrations within the range shown to have enzyme-perturbing effects. In *T. maxima* and *T. squamosa* of the sizes we studied, the mantle averages about  $0.83\ \text{ml}$  water per gram wet weight (our unpublished data). Thus, if we assume that the DMSP in mantle is entirely dissolved and distributed evenly in tissue water, the mean concentration of DMSP in the tissue water is over  $40\ \text{mM}$ . On the basis of the chemical structural properties of DMSP and the preferential hydration model, Nishiguchi and Somero (1992) argue that DMSP, like dimethylsulfoxide, may be toxic to cells and may denature proteins at high physiological temperatures. In warm tropical waters and especially in shallows where solar heat-

ing can occur, the high DMSP concentrations of tridacnids may be stressful by-products of extreme exploitation of phototrophic nutrition (Klumpp and Griffiths, 1994). Tridacnids may require specializations of metabolic chemistry to reduce or tolerate enzyme-perturbing and other toxic effects of their high DMSP concentrations. Interspecific differences in such specializations might help explain differences in growth rates and energetics that have previously remained enigmatic (Klumpp and Griffiths, 1994).

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