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## Supporting Online Material for

### **Dimethylsulfoniopropionate as a Foraging Cue for Reef Fishes**

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#### **This PDF file includes:**

Materials and Methods  
References

## **Supporting Online Material**

### **Methods**

**Experimental design.** DMSP and control trials were performed between 31 October and 15 November 2006 at four reef sites. Paired trials (DMSP and control) were performed at each site, typically on sequential days. For each pair, releases began at the same time each day (within 36 min; avg  $\pm$  s.d.,  $18.4 \pm 11.8$  min), starting in either the morning or the afternoon (10:30-16:04 hrs). Release order was randomized. Paired trials performed at the same site were separated by at least a week.

**DMSP.** We released  $10^{-7}$  M DMSP to approximate biogenic mid-water concentrations documented during plankton blooms (*SI*). This source concentration was at least ten times higher than DMSPt background levels we previously measured in the Netherlands Antilles (6.0 – 57.0 nM). DMSP stock solutions ( $1.9 \times 10^{-3}$  M in distilled water, Chemische Laboratoria, Rijksuniversiteit Groningen, Groningen, The Netherlands) and distilled water controls were prepared and distributed into coded 1.5 mL microcentrifuge tubes, so that trials could be done blind. Tubes were immediately frozen ( $-18.0^{\circ}\text{C}$ ) until ~1 hour before use. After stock solutions were added to distilled water, carboys were sealed, vigorously shaken, tied together in pairs, and towed out to the edge of the downward sloping reef (9-14 m depth). Two pairs of carboys were anchored 2-3 m apart, where they floated mid-water (7-12 m depth). To extend the release time, we staggered opening the pairs of carboys such that one pair was opened at time zero, followed by a second pair at 20 min. Pilot trials using fluorescein dye indicated that equilibration with ambient seawater occurred within 40-60 min of opening the carboys, suggesting that nearly all solution was evacuated in that timeframe. DMSP concentrations in water

samples (five replicates per trial) taken at 55 min three meters up and down-current of DMSP releases were  $6.12 \pm 0.40$  nM and  $6.41 \pm 0.20$  nM, respectively (for methods see S2).

**Fish counts.** Immediately before carboys were opened, “background” fish counts were taken by two observers on SCUBA, hovering at 10 m depth, perpendicular to the current to avoid interfering with the odor plumes. Stationary point counts were taken of mid-water planktivores (see text) in the water column, directly over and up to 10 m downstream of the carboys at 10-min intervals, to 60 min. For analysis, both observers’ counts were averaged for each 10 min interval.

**Analyses.** We used a two-way ANOVA to examine the difference in background fish counts among sites and treatments (JMP 4.0.2, SAS Institute, Inc.). Subsequent analysis used a Friedman’s test for non-parametric repeated-measures modified for multiple cases per cell (S3). Boga counts could not be analyzed due to the multiple zero counts during controls, so raw data are shown without statistical analysis.

## References

- S1. J. W. H. Dacey, G. M. King, P. S. Lobel, *Mar. Ecol. Prog. Ser.* **112**, 67-74 (1994).
- S2. J. L. DeBose, G. A. Nevitt, *Mar. Freshw. Res.* **58**, 720-724 (2007).
- S3. L. A. Marascuilo, M. McSweeney, *Nonparametric and distribution-free methods for the social sciences* (Brooks/Cole, Monterey, 1977).

## **ONLINE SUMMARY**

**Coral reefs resemble “islands” of productive habitat where fishes aggregate, forage and spawn. While it has been suggested that some reef fishes use biogenic chemicals as aggregation cues, specific chemicals have not been identified.**

**Dimethylsulfoniopropionate (DMSP), a secondary metabolite of many marine algal species, is released during foraging by higher order consumers. DMSP has been studied intensively for its role in oceanic sulfur cycles and global climate regulation, but its ecological significance to marine fishes is unknown. Here we present evidence that planktivorous reef fishes will aggregate to experimental deployments of DMSP over coral reef habitats in the wild.**