The following supplement accompanies the article

AS WE SEE IT

Use of clove oil in collecting coral reef fishes for research

D. R. Robertson[1,*], W. F. Smith-Vaniz[2]

[1]**Smithsonian Tropical Research Institute, Balboa, Republic of Panamá**
[2]**Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA**

*Email: drr@stri.org

Marine Ecology Progress Series 401:295–302 (2010)

The 3 studies on the effects of clove oil on corals (Mulochau & Durville 2004, Frisch et al. 2007, and Boyer et al. 2009) cited a total of 9 field studies in which clove-oil solution (COS) was used to collect coral reef fishes. These include 7 studies listed by Boyer et al. (2009) as involving repeated sampling of sites using COS, on which they based their protocol of repetitive treatments of live corals with COS. Below we summarize the use of COS in these 9 studies, as well as the use of COS in 8 others, together with information on the effects of COS usage on corals as noted by the authors of these studies

1. Ackerman & Bellwood (2002) used ALCOS (23% clove oil in 98% ethanol) to make confined-assemblage collections. Each site was treated with sufficient COS solution to provide an overall maximum potential concentration of ~0.13 ppt clove oil under a netting tent. The COS took ~5 min to apply, and dispersed completely within 15 to 20 min. Although Ackerman & Bellwood (2002) were cited by Boyer et al. (2009) as having resampled the same sites 3× at yearly intervals, they actually sampled each of 5 sites only once, with that sampling being spread over 3 consecutive years. The total area (live coral plus other substrata) that was treated with COS in this study was 17.5 m^2. No discoloration, bleaching or mortality of corals in the treated areas was noted during return visits made to the same general area of reef within a week of the original application of COS, either by the principal author or by other researchers working in the immediate area (J. Ackerman pers. comm., April 2009).

2. Arvedlund et al. (2006) used ALCOS (10% clove oil in 96% ethanol) to catch juveniles of a wrasse living in sea anemones. They used ~100 and ~500 ml of ALCOS to collect 4 and 11 fish, respectively, from 2 individual anemones. This large quantity of COS was needed because the fish were very agile, and the anemones were in an area of strong currents that rapidly flushed the COS away as it was applied. The authors revisited the anemones 1 wk after the collection and noted no visible adverse effects of the COS application. The total area of anemones that was treated with COS in this study was 0.14 m^2 (M. Arvedlund pers. comm., April 2009).

3. Depczynski & Bellwood (2004) (see also Depczynski & Bellwood 2003). Although Boyer et al. (2009) indicated that this study involved 7 repeated collections at 14 d intervals, it actually involved collections of fish at 84 different 0.4 m^2 sites. These sites, which were distributed in 3 areas, contained 4 microhabitats, 2 of which included live coral. In addition, 25 sites composed of dead coral rubble were used to assess site fidelity of tagged fish, with each site being resampled once, 2 d after the initial catch-and-tag operation. ALCOS (20% clove oil in 80% ethanol) was used for these confined-assemblage collections, with each site being treated with COS that was injected under a fine-mesh netting tent (0.4 m^2 basal area, 0.3 m height). The maximum potential concentration of clove oil under each tent was ~0.53 ppt, and collecting commenced 1 min after COS application. The total area of substrate that was sampled during this study was 47 m^2, ~10 m^2 of which represented live coral. No observations were made on the status of corals in the sampling sites following these collections (M. Depczynski pers. comm., April 2009).

4. Munday (2004) (see also Munday 2001a,b, 2002, Munday et al. 2006) studied gobies that are obligate inquilines living within finely branched, live *Acropora* corals. ALCOS (12 to 25% clove oil in 50 to 75% ethanol) was used in several different experiments that involved removals of fish from coral colonies, then resampling the same colonies once, 4 mo later. During these collections, individual coral colonies that were 10 to 40 cm in diameter were typically treated with ~10 ml of ALCOS delivered from a squirt bottle. No obvious discoloration or other damage to colonies that were sampled repeatedly in this way was noted. Munday (2002) and Munday et al. (2006) sampled 16 and 10 m^2 of live coral, respectively, with COS (P. Munday pers. comm., May 2009).

5. Shima et al. (2006, 2008) used AQCOS (10% clove oil in seawater) to collect small juveniles of a wrasse (*Thalassoma hardwicke*), the preferred microhabitat of which is live *Pocillopora* corals. Shima et al. (2006) made collections on 60 small patch reefs (4.9 m^2 average size) that contained an average of 4% live *Pocillopora* cover. Shima et al. (2008) made collections on 48 small patch reefs (11.2 m^2 average size) that contained an average of 3% live *Pocillopora* cover. Juvenile fish densities averaged 0.6 m^{-2}. Collecting regimes ranged from a single collection on any reef (Shima et al. 2006), to many repeat collections on the same patch reefs and, often, in the same coral colonies, sometimes with multiple collections on the same reef on the same day (Shima et al. 2008). Boyer et al's (2009) estimate of 3 repeat collections at 12 d intervals was probably conservative in the case of the Shima et al. (2008) study. Fish were caught using COS delivered by squirt bottle, and aquarium hand nets. Because this wrasse species is highly active and difficult to catch, numerous small, scattered doses of COS were often used on different parts of a single patch reef, with roughly 50 to 100 ml of COS being needed to catch a single fish. No unusual levels of discoloration or bleaching of corals were noted on the sampled reefs during either study, although corals that were exposed to COS were not monitored closely (J. Shima pers comm., April 2009). The 2 studies involved various regimes of COS sampling of 11.8 m^2 (Shima et al. 2006) and 16.7 m^2 (Shima et al. 2008) of live *Pocillopora*.

6. Valles et al. (2006) repeatedly sampled the same sites either at daily or 10 d intervals, as Boyer et al. (2009) indicated. However, rather than live coral, these sites consisted of dead coral rubble in containers of a standard size. The authors used <100 ml of ALCOS (8% clove oil in 22% isopropanol) to treat 36 l of rubble $container^{-1}$, providing a brief application of <0.2 ppt of dispersed clove oil to the rubble in a netting bag, which is equivalent to making a confined-aggregation collection in a porous tent (H. Valles pers comm., April 2009). The total surface area of substrata in the containers that were treated with COS was 10.1 m^2.

7. Vigliola et al. (2007) (see also Vigliola & Meekan 2002) were cited by Boyer et al. (2009) as having made 14 collections of *Neopomacentrus filamentosus* (a reef fish that associates with live corals) at daily intervals, with the implication of repeated sampling of the same corals. However, sampling was actually done only 7× at monthly intervals, with each such collection episode involving many different coral colonies spread over a large reef. Any (highly unlikely) repeat sampling of individual coral colonies would have been at monthly intervals. Further, captures of the smallest fish that were most closely associated with corals were made without the use of COS and, when used, ALCOS (10% clove oil in 70% ethanol) was released as a small cloud that drifted into a group of larger fish hovering above a coral colony rather than directly into the coral (L. Vigliola pers. comm., April 2009). Thus, no live coral was directly treated with COS during this study.

8. Whiteman & Côté (2002a,b, 2003, 2004a,b) used ALCOS (10% clove oil in 90% ethanol) to collect cleaner gobies (*Elacatinus* spp.) that lived directly on the surface of live massive corals (*Siderastrea*, *Montastrea*) and sponges. As the study species was easy to capture, only milliters of ALCOS were required to capture each fish. During one series of experiments (Whiteman & Côté 2003), 2 sequential collections separated by ~7 d were made on the same set of coral heads. During these studies, some small patches of temporary discoloration were observed on one massive coral (*Siderastrea siderea*) colony that could have been due to ALCOS use, but there was no bleaching (whitening) or coral mortality (E. Whiteman pers comm., April 2009).

9. Wilson (2005) used ALCOS (2% clove oil in 70% isopropanol) to collect cleaner gobies that lived directly on the surface of live massive (*Montastrea*) coral heads. She resampled individual, relatively small (~20 to 40 cm diameter) coral heads at 2 mo intervals twice over a 5 mo period. A few ml (<10) of COS was usually sufficient to catch individual fish, with rarely as much as >200 ml being gradually applied over the surface of a single coral head to catch multiple fish. Individual polyps likely received multiple COS treatments during such resampling activity. Neither damage to corals nor coral mortality were noted during these experiments (J. Wilson pers. comm., April 2009). The 28 live coral heads to which COS was applied in this study had a total surface area of 9.45 m^2.

In addition, we obtained the following information on COS usage in 8 other studies:

10. Bellwood et al. (2006) used ALCOS (23% clove oil in 98% ethanol) to make confined-assemblage collections of cryptic fishes on small patch reefs. Between 1993 and 2004, 2 to 4 reefs were sampled annually, with each sampling site being individually enclosed under a fine-mesh netting tent (3.5 m^2 basal area, 2 m^3 volume). Sufficient COS was injected into each tent to provide an overall maximum potential concentration of ~0.13 ppt clove oil. The COS took ~5 to 10 min to apply, and dispersed completely within 5 to 10 min. During the 12 yr of this study, 35 sites were sampled, with a total area of 112 m^2. Following a repeat set of collections in 2009, the sampled sites were revisited 1 d later. No discoloration or bleaching of corals in excess of that on reefs in the immediate vicinity of the sampled reefs was noted (D. Bellwood pers. comm., April 2009).

11. Castellanos-Galindo et al. (2005) and Castellanos-Galindo & Giraldo (2008) used ALCOS (12% clove oil in 95% ethanol) to make confined-assemblage collections in 10 small (4.9 m^3 combined volume) tide pools on a rocky shore on the Pacific coast of Colombia. Dispersed COS in the tide pools contained

~0.01 ppt clove oil. Exposure times before flushing by the incoming tide ranged from <1 h for the lowest pools to 2 to 4 h for the highest pools (G. Gastellano-Galindo pers. comm., April 2009).

12. Depczynski & Bellwood (2005, 2006) made ALCOS collections (at a different location from that of Depczynski & Bellwood 2003, 2004) for their studies on the effects of wave stress on habitat use of small, cryptic reef fishes (Depczynski & Bellwood 2005), and the demography of these species (Depczynski & Bellwood 2006). They used the same tenting and COS application methods as those of Depczynski & Bellwood (2003, 2004). For the habitat-use study, they sampled 216 sites (0.4 m^2 each) that spanned 5 different microhabitats with varying levels of live coral cover (5 to 50%). Eighty-six of the 0.4 m^2 sites sampled in this study (i.e. ~16 m^2) represented live coral. For the 2006 demography study, data were used from fish that were collected in the 2005 study, plus collections from an additional 12 sites (1 m^2 each) in rubble and sand habitat, including <1 m^2 of live coral. No observations were made on the status of corals in the sampling sites following either series of collections (M. Depczynski pers. comm., April 2009).

13. González-Cabello & Bellwood (2009) made non-repetitive, confined-assemblage collections using ALCOS (20% clove oil in 80% ethanol) that was injected under small, conical (0.4 m^2 basal area, 0.3 m high), nonporous plastic tents. Each collection involved a 3 min exposure to a diluted solution that would have produced an initial potential maximum concentration of ~0.53 ppt clove oil. Collections were made in 4 microhabitats: live *Pocillopora* coral colonies, plus 3 rocky microhabitats. No information is available on responses of corals to ALCOS as the sites were not revisited (A. González-Cabello pers. comm., April 2009). The total area that was sampled using COS was 16 m^2 (including 4 m^2 of live coral) at each of the west and east Pacific sites.

14. Marnane (2000) used ALCOS (10% clove oil in 50% ethanol) to collect cardinalfishes on coral reefs. COS was delivered to individual fish both in open water and under enclosure tents. Doses delivered in open water varied from a single squirt of <10 ml for individuals or small groups of small, sedentary species, to 2 to 3 such doses spread over a larger area for larger, more mobile species. Cryptic species hiding within a coralline substrate matrix were collected with 0.5 to 1.0 l of ALCOS that was squirted under a plastic sheet (~4 m^2 basal area, ~2 m^3 partly enclosed volume, producing an overall potential maximum concentration of 0.025 to 0.05 ppt clove oil), with the sheet being kept in place for 1 to 5 min before removal to allow collecting. Most of the apogonids that were tagged in these studies were resighted regularly within 10 to 50 cm of their original capture position and longer-lived species were recaptured at the same sites up to 3× over a 3 yr period. Hence, many clusters of coral polyps were repeatedly exposed to COS during this period. The only adverse reactions by corals to the COS treatments that were noted during this study were slight, temporary discoloration in some cases, probably due to temporary polyp retraction (M. Marnane pers. comm., April 2009).

15. Wen et al. (2005) used ALCOS (clove oil in 50% ethanol) to make confined-assemblage collections in 3 small (<3 m^3 total combined volume) tide pools on a coral reef in Taiwan. ALCOS was applied to achieve an overall concentration of 0.1 ppt clove oil in each pool.

16. Wilson (2000, 2004) & Wilson et al. (2001) used ALCOS (5 to 10% clove oil in 90% ethanol) to collect a hole-dwelling blenny from both live- and dead-coral microhabitats on a coral reef. Fish were collected individually, with ≤10 ml of COS being applied to any single hole or small crevice. No repeat collections were made at the same sites, and no adverse reactions by corals were noted on return visits to the collection areas (S. Wilson pers. comm., April 2009).

17. Zapata & Herrón (2002) used ALCOS (10% clove oil in 95% ethanol) to collect newly settled reef fishes at 2 locations on the Pacific coast of Colombia, one of them being a coral reef. Doses of ~10 ml of COS were delivered from a squirt bottle to individual fish collected from a variety of microhabitats, including live corals. As sites were not revisited, there is no record of any adverse responses by corals to COS usage (F. Zapata pers. comm., April 2009).

LITERATURE CITED

Ackerman JL, Bellwood DR (2002) Comparative efficiency of clove oil and rotenone for sampling tropical reef fish assemblages. J Fish Biol 60:893–901

Arvedlund M, Iwao K, Brolund TM, Takemura A (2006) Juvenile *Thalassoma amblycephalum* Bleeker (Labridae, Teleostei) dwelling among the tentacles of sea anemones: a cleanerfish with an unusual client? J Exp Mar Biol Ecol 329:161–173

Bellwood DR, Hoey AS, Ackerman JL, Depczynski M (2006) Coral bleaching, reef fish community phase shifts and the resilience of coral reefs. Glob Change Biol 12:1587–1594

Boyer SE, White JS, Stier AC, Osenberg CW (2009) Effects of the fish anesthetic, clove oil (eugenol), on coral health and growth. J Exp Mar Biol Ecol 369:53–57

Castellanos-Galindo G, Giraldo A (2008) Food resource use in a tropical eastern Pacific tidepool fish assemblage. Mar Biol 153:1023–1035

Castellanos-Galindo GA, Giraldo A, Rubio EA (2005) Community structure of an assemblage of tidepool fishes on a tropical eastern Pacific rocky shore, Colombia. J Fish Biol 67:392–408

Depczynski M, Bellwood DR (2003) The role of cryptobenthic reef fishes in coral reef trophodynamics. Mar Ecol Prog Ser 256:183–191

Depczynski M, Bellwood DR (2004) Microhabitat utilization patterns in cryptobenthic coral reef fish communities. Mar Biol 145:455–463

Depczynski M, Bellwood DR (2005) Wave energy and spatial variability in community structure of small cryptic coral reef fishes. Mar Ecol Prog Ser 303:283–293

Depczynski M, Bellwood DR (2006) Extremes, plasticity, and invariance in vertebrate life history traits: insights from coral reef fishes. Ecology 87:3119–3127

Frisch AJ, Ulstrup KE, Hobbs JPA (2007) The effects of clove oil on coral: an experimental evaluation using *Pocillopora damicornis.* J Exp Mar Biol Ecol 345:101–109

González-Cabello A, Bellwood DR (2009) Local ecological impacts of regional biodiversity on reef fish assemblages. J Biogeogr 36:1129–1137

Marnane MJ (2000) Site fidelity and homing behavior in coral reef cardinalfishes. J Fish Biol 57:1590–1600

Mulochau T, Durville P (2004) Effets de solution d'essence de girofle-éthanol sur le corail *Pocillopora verrucosa.* Rev Ecol Terre Vie 59:425–432

Munday PL (2001a) Interspecific competition and coexistence in a guild of coral-dwelling fishes. Ecology 82:2177–2189

Munday PL (2001b) Fitness consequences of habitat use and competition among coral-dwelling fishes. Oecologia 128: 585–593

Munday PL (2002) Bi-directional sex change: testing the growth-rate advantage model. Behav Ecol Sociobiol 52: 247–254

Munday PL (2004) Competitive coexistence of coral-dwelling fishes: the lottery hypothesis revisited. Ecology 85: 623–628

Munday PL, Cardoni AM, Syms C (2006) Competitive growth regulation in coral-dwelling fishes. Biol Lett 2: 355–358

Shima JS, Osenberg CW, St Mary CM, Rogers L (2006) Implication of changing coral communities: Do larval traits or habitat features drive variation in density-dependent mortality and recruitment of juvenile reef fish? Proc 10th Int Coral Reef Symp, Okinawa, p 226–231

Shima JS, Osenberg CW, St Mary CM (2008) Quantifying site quality in a heterogeneous landscape: recruitment of a reef fish. Ecology 89:86–94

Valles H, Kramer DL, Hunte W (2006) A standard unit for monitoring recruitment of fishes to coral reef rubble. J Exp Mar Biol Ecol 336:171–183

Vigliola L, Meekan MG (2002) Size at hatching and planktonic growth determine post-settlement survivorship of a coral reef fish. Oecologia 131:89–93

Vigliola L, Doherty PJ, Meekan MG, Drown DM, Jones ME, Barber PH (2007) Genetic identity determines risk of post-settlement mortality of a marine fish. Ecology 88: 1263–1277

Wen KC, Shao KT, Ho LT, Chen LS (2005) A comparison between clove oil and rotenone for collecting subtropical intertidal fishes. J Fish Soc Taiwan 32:29–39

Whiteman EA, Côté IM (2002a) Cleaning activity of two Caribbean cleaning gobies: intra- and interspecific comparisons. J Fish Biol 60:1443–1458

Whiteman EA, Côté IM (2002b) Sex differences in cleaning behavior and diet of a Caribbean cleaning goby. J Mar Biol Assoc UK 82:655–664

Whiteman EA, Côté IM (2003) Social monogamy in a Caribbean cleaning goby: Ecological constraints or economic advantage? Anim Behav 66:281–291

Whiteman EA, Côté IM (2004a) Individual differences in microhabitat use in a Caribbean cleaning goby: a buffer effect in a marine species. J Anim Ecol 73:831–840

Whiteman EA, Côté IM (2004b) Dominance hierarchies in group-living cleaning gobies: patterns of interaction and foraging consequences. Anim Behav 67:239–247

Wilson SK (2000) Trophic status and feeding selectivity of blennies (Blenniidae: Salariini). Mar Biol 136:431–437

Wilson SK (2004) Growth, mortality and turnover rates of a small detritivorous fish. Mar Ecol Prog Ser 284: 253–259

Wilson JA (2005) Age class interactions in a marine goby, *Elacatinus prochilos* (Bohlke and Robins, 1968). J Exp Mar Biol Ecol 327:144–156

Wilson SK, Burns K, Codi S (2001) Sources of dietary lipids in the coral reef blenny *Salarias patzneri.* Mar Ecol Prog Ser 222:291–296

Zapata FA, Herrón PA (2002) Pelagic larval duration and geographic distribution of tropical eastern Pacific snappers (Pisces: Lutjanidae). Mar Ecol Prog Ser 230:295–300