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AS WE SEE IT

Use of clove oil in collecting coral reef fishes for research

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ABSTRACT: Managers need accurate and relevant information about potential adverse environmental effects of scientific collecting when considering research proposals and permits. Clove oil has recently come into use in scientific fish-collecting. While several short-term experimental studies on clove oil's effects on corals have found negative effects, these were in response to heavier dosages than are typically used by researchers to collect fishes. Thus, the available evidence suggests that the small amounts of this oil that are normally applied during such collections rarely visibly stress corals. Experiments are needed to test for negative effects of actual scientific collecting with clove oil to clarify the real-world consequences of its use on coral survivorship, growth and reproduction at ecologically significant scales. When managers are assessing proposals for research that requires collecting fish, they should place the attendant environmental costs in perspective, and weight them against the relative value of the potential research results. Coral reefs occupy enormous areas of the tropics, and corals are also common in other habitats. Coral populations are often highly dynamic, possess strong powers of regeneration, and recover from repeated effects of temporary, large-scale natural events (hurricanes, floods, volcanic eruptions, tsunamis). The relatively small numbers of researchers collecting reef fishes with clove oil do so only intermittently, in areas of a few m^2 per project, and at sites that are widely dispersed throughout the tropics. Any negative effects of such tiny, brief, scattered collections are inconsequential relative to the effects of acute and chronic large-scale natural and human-induced stresses on coral populations, and to the regenerative capabilities of corals.

KEY WORDS: Clove oil · Anesthetic · Coral reef fishes · Collecting · Management

Introduction

Appreciation of the beauty and diversity of life on coral reefs has grown considerably over the past quarter century, and has penetrated so widely in human society that corals now represent iconic organisms. This view has been strengthened by growing concern over the dramatic, pan-tropical declines in coral populations that have occurred due to the chronic effects of warming-induced bleaching, coral diseases, pollution, development and overfishing (e.g. Hughes et al. 2003, Carpenter et al. 2008). One consequence is the perception that corals and coral reefs need extraordinary protective measures due to their 'fragility'. Individual coral colonies are certainly fragile to the extent that they are easily broken and physically damaged. However, notwithstanding the human-induced global declines of coral reefs, coral populations and reefs are naturally highly dynamic entities that for eons have rebounded from the dramatic short-term effects of major natural environmental events, such as hurricanes, floods, volcanic eruptions and tsunamis (Dollar & Tribble 1993, Tomascik et al. 1996, Connell et al. 1997, Done 1997, Lugo et al. 2000, Halford et al. 2004, Bellwood et al. 2006, Game et al. 2008, Veron 2008). They also recover from short-term, human-induced major disruptions, such as those produced by atomic explosions (e.g. Richards et al. 2008).

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Increasing environmental awareness and governmental control of marine resources such as coral reefs have led managers to become more reluctant to allow research collecting of marine organisms, including coral reef fishes. However, virtually all research on the biology of fishes requires some collecting activity. Much research involves live fishes; catch-tag-release enables the tracking of individuals, while manipulation of individuals, groups and abundances in carefully designed experiments is the only scientifically valid way to determine the effects of specific processes. Fish specimens are essential for studies on biogeography, ecology (microhabitat usage, diet), demography (age, growth and longevity, and the effects of environmental variation and fishing on those demographic parameters), reproductive biology (sexual identity and condition, sex ratios, maturation, reproductive history), physiological effects of pollution, and genetic studies (e.g. barcoding) of the identification of species and the mechanisms of evolution. Augmenting this information is essential for gaining an understanding of the biology of coral reef fishes that can provide the basis for effective management and conservation. Management decisions should be based on the best data available, and research collecting is essential for expanding the scope and availability of such information (Robertson & Smith-Vaniz 2008).

Clove oil, which acts as an effective and rapidly acting anesthetic on fishes (Soto & Burhanuddin 1995, Munday & Wilson 1997, Keene et al. 1998, Griffiths 2000), has come into usage in research collecting of live reef fishes in various parts of the world over the last decade (Erdmann 1999). This oil is as effective as other anesthetics that are commonly used by scientists to collect fishes in the field (Munday & Wilson 1997). Clove oil is obtained by distillation of parts of the clove plant *Eugenia caryophyllata*, with the main active ingredient (eugenol) representing ~70 to 98% of the content of the oil (Harper 2003). Accumulated experience with its usage indicates that most research collecting of live reef-fish with anesthetics can be done using this oil. In typical applications, it is thought to be minimally destructive to marine organisms, including target species (Erdmann 1999). Clove oil has other advantages, as it has a long history of safe human usage as an analgesic and antiseptic, and cloves themselves are a foodstuff. If the use of clove oil in research collecting of coral reef fishes typically has few adverse side effects, then it could replace other anesthetics that appear to be more hazardous to human users and more detrimental to both nontarget and target organisms (Erdmann 1999).

Three recent studies assessed the potentially adverse affects of clove oil use (in research collecting of reef fishes) on live corals. In this paper, we (1) summarize how researchers in 17 published studies used clove oil to collect coral reef fishes, and indicate what they noticed about adverse effects of clove oil usage on corals; (2) examine the methods and conclusions of the 3 formal studies on the effects of clove oil on live corals; and (3) indicate in general terms the design features of future experiments that should provide more useful and relevant information.

Finally, we address an issue that, while important for management, was ignored by these 3 studies on the effects of clove oil on corals: How biologically significant are any adverse affects of such research collecting for the maintenance and protection of coral reefs?

Use of clove oil for sampling reef fishes

Here, we present an overview of differing patterns of use of clove-oil solution (COS) in 17 studies. A summary of each study, and any observations by its authors on adverse effects of COS use on corals, is contained in an online supplement available at www.int-res.com/articles/suppl/m401p295_app.pdf.

COS is used in several ways by researchers to collect fishes that live in close association with reef substrata. Clove oil concentrations in COS range from 2 to 28%. Sometimes, the oil is mixed with seawater (aqueous clove-oil solution, AQCOS). More typically (16 of 17 studies), it is dissolved in alcohol (alcoholic clove-oil solution, ALCOS) because it is relatively immiscible with water.

When used to catch live individual fish (or small groups of fish), COS is squirted by hand pressure from the nozzle of a soft plastic bottle containing ~500 ml of solution. Different bottle designs either maintain the concentration of the COS until it is all used, or allow it to be gradually diluted as the original contents are replaced with seawater. The objective is to briefly envelope the fish in a cloud of COS, to calm and disorient it so that it can be scooped up with an aquarium hand net (authors' pers. obs.). During this process, a small dose (typically ~10 ml) is squirted at a fish, although multiple doses scattered over a patch reef or coral colony may occasionally be needed to catch individuals of highly active, elusive species. Delivered in this way, COS disperses very rapidly in unconfined spaces. For example, Boyer et al. (2009) found that water collected within 10 cm of the site of application of relatively large quantities of AQCOS or ALCOS (120 ml) contained <1% of the delivery dose of clove oil within 5 s of application, and virtually nothing 25 s after application. Such rapid dispersion is consistent with our personal experiences using ALCOS to catch individual fishes in open water. As Boyer et al. (2009) point out, however, dispersion will be slower in more

confined spaces. The density of alcohol is ~3/4 that of seawater, which should assist dispersion from such sites.

In addition, ALCOS has been used for larger-scale collections in which small tents that are constructed either of netting or plastic sheeting were set up over a sampling site to slow the dispersion of COS and prevent the escape of fishes. In such confined-assemblage collections, COS is slowly squirted under the tent over a 5 to 10 min period, and the tent is kept in place for a further 1 to 3 min before collecting begins. Due to leakage, COS injected under a tent immediately starts to dilute and disperse, and typically disperses completely within 5 to 20 min after removal of the tent. Maximum potential initial dosages of clove oil under tents have ranged from 0.13 to 0.53 ppt in different studies (assuming that all the COS was evenly dispersed under the tent, and remained there) (see online supplement), but actual maximum dosages would be lower due to leakage, and much lower in the case of porous netting tents.

Finally, confined-assemblage collections have also been made in small tide pools that are exposed during low tide. In these studies, sufficient ALCOS was dispersed throughout a pool to provide overall clove-oil concentrations ranging from 0.01 to 0.1 ppt (see online supplement). Unlike the situation with COS applied under tents in open water, the initial concentration of COS in a tide pool was maintained for 1 to 4 h until the pools were flushed by the rising tide.

Effects of clove oil on corals

There have been 3 formal experimental studies on the effects of COS on corals: Mulochau & Durville (2004), Frisch et al. (2007), and Boyer et al. (2009). These focused primarily on the use of COS in research collecting of reef fishes (as opposed to commercial collecting of aquarium fishes) when providing a rationale for their experiments. As measures of stress on corals, the authors used loss of pigmentation leading to discoloration and bleaching (whitening), death, reductions in photosynthetic capability, and reductions in growth.

(1) Mulochau & Durville (2004) tested responses of a densely branching *Pocillopora* coral to aquarium baths of diluted ALCOS (20% clove oil, 80% ethanol). These included either (1) a single bath containing 2 ppt clove oil for each of 4 durations (1, 2.5, 5 and 10 min), or (2) 5 baths each of 0.2 ppt clove oil, each bath separated by a 3 d interval for each of the same 4 durations as in the single bath treatment. After treatment, corals were returned to flow-through aquaria and observed daily for 1 mo. For the single bath in 2 ppt clove oil, 1 of 4 colonies discolored after 1 min immersion, 2 of 4 after 2.5 min, and all 4 after 5 min; all 4 colonies were entirely bleached after 10 min. Five baths of 0.2 ppt clove oil produced no response from corals after 1 min immersion, 1 of 4 colonies showed discoloration after 2.5 or 5 min, and 2 of 4 discolored after 10 min immersion. These color changes occurred within hours to days of the treatment, and no discolored or bleached corals recovered normal coloration during the month of post-treatment observations (T. Mulochau pers. comm., April 2009). Mulochau & Durville (2004) concluded that corals were less affected by a series of small doses of COS than by a single larger dose equal in size to the sum of the small doses.

Positive aspects of the study: entire colonies rather than fragments were used, and these were allowed a long acclimation period (8 wk prior to the start of the experiments).

Limitations on real-world relevance of study: aquarium experiments were used, which could have reduced the resistance of corals, which are known to be more stressed in aquaria than in the field (Frisch et al. 2007, Willis 2004). The level of replication was relatively low (4 coral colonies per bath, 4 controls), and results were not subjected to statistical analyses. Concentrations of clove oil that produced strong adverse reactions from corals were distinctly higher than those generally used in confined-assemblage collections in the field.

(2) Frisch et al. (2007) used both laboratory and field tests to examine responses of a finely branched *Pocillopora* species to ALCOS.

Laboratory experiments: 4 d after being broken off a colony, coral fragments were immersed in a bath of 1 of 18 exposure treatments: 0.05, 0.5 and 5 ppt clove oil (as ALCOS); 0.5, 5 or 50 ppt ethanol in seawater; plus a seawater control, with either 1, 10 or 60 min exposure during each treatment. Corals were then returned to their original recirculating aquarium and monitored for 7 d. Exposure to 0.05 ppt clove oil for 1 to 60 min had no effect on coral color; 0.5 ppt clove oil exposure for 60 min killed coral fragments within 2 d, 10 min exposure produced discoloration within 2 to 3 d, and 1 min exposure had no effect on coral color; 5 ppt clove oil exposure for 1 min or more killed all corals within 1 to 2 d. The ethanol-only treatments had no effect on coral color. Photosynthetic efficiency was reduced in all treatments except the 0.05 ppt clove oil and alcohol-only treatments.

Field experiments: these involved 3 treatments, 10 or 100 ml of full-strength ALCOS (100 ppt clove oil, 900 ppt ethanol), or 100 ml of 100% ethanol sprayed at close range over a period of 1 min into the center of the tight matrix of a coral colony. Corals were then moni-

tored for 63 d. Compared to the controls, exposure to either 100 ml of ethanol or 10 ml of ALCOS produced no increased discoloration, nor partial colony mortality. Exposure to 100 ml of ALCOS, however, produced discoloration in the center of the colony within 2 d, and partial mortality (a patch ~5 cm in diameter) at the point of application ~7 d later.

Positive aspects of study: weaker, shorter-duration test applications of COS in the laboratory were within the range of concentrations used in confined-assemblage collections under tents (see previous section). The smaller of the 2 amounts of concentrated COS (10 ml) used in the field test was also within the range of amounts used in real-world research collecting (see previous section). Both laboratory and field experiments were reasonably well replicated (n = 9 treatment^{-1} for laboratory experiments, 7 treatment^{-1} for field experiments), and conclusions were based on statistical analyses. Field tests used whole adult coral colonies, and changes in coral status were monitored for an extended period.

Limitations on real-world relevance of study: (1) in the laboratory experiments, coral fragments were used that allowed only short acclimation periods (4 d) before treatments began. In contrast, Jones (1997) allowed 14 to 20 d for fragment acclimation in the field, by which time fragments had produced a small skirt of tissue-covered skeleton at the broken base, while Mulochau & Durville (2004) allowed 8 wk for small colonies in aquaria. (2) COS treatments that had adverse effects on corals involved substantially higher doses and levels of exposure than would normally occur in field collections of fishes.

(3) Boyer et al. (2009) tested the effects of COS on fragments of coral belonging to 1 species in each of 3 genera (*Pocillopora*, *Acropora* and *Porites*) in the field. Tips of adult colonies were attached to a concrete block, and allowed to acclimate for 1 wk prior to the experimental treatments. Four treatments were used: 3 of AQCOS (70, 140 or 280 ppt clove oil), and 1 of ALCOS (140 ppt clove oil, 760 ppt ethanol), plus a seawater control. Each exposure involved 120 ml of COS being squirted directly onto the group of 3 fragments sharing a block. These treatments were repeated 5× at weekly intervals. To measure adverse effects of COS application, these authors used (1) the proportion of weeks in which any part of a colony was discolored, and (2) the reduction in growth of these fragments measured 1 wk after the last treatment. Increased discoloration and reduced growth were found in all treatments except 70 ppt AQCOS. No coral mortality was mentioned.

Positive aspects of study: field experiments were used, and an attempt was made to control for genetic variability by using fragments from different colonies. Corals from various genera were tested. Conclusions were based on statistical analyses.

Limitations on real-world relevance of study: only coral fragments were used (the response of a fragment may differ from that of an entire colony that has only part of its surface treated with COS); growth was measured over a very short period; a very short acclimation period was used prior to the start of the experiments; and there were low levels of replication (3 fragments species^{-1} treatment^{-1}, plus 3 controls). In general, the levels of exposure used (large quantities of moderate to high strength COS applied at close range, with frequent reapplications) were substantially higher than those used in almost all real-world scientific collecting.

Effects of clove oil on corals vs. actual usage patterns

Most researchers collecting reef fishes with COS have used ALCOS (16 of 17 studies). Concentrations varied widely between 2 and 23% clove oil combined with 25 to 90% ethanol or, occasionally, isopropanol. Collecting with COS has involved exposure of live corals to COS in most (13 of 17) studies.

During individual-fish collections, concentrated COS was delivered in small quantities (≤10 ml) at any particular point on a coral. Large doses (~100 ml) of concentrated COS were applied to a small cluster of coral polyps in only 1 case (Shima et al. 2008, see online supplement), and repeat treatments of groups of polyps at short intervals occurred (infrequently) only in Shima et al. (2008).

Corals can display obvious visible signs of stress in response to COS application. With increasingly stronger concentrations or duration of exposure, these include partial discoloration, complete discoloration (bleaching), and death. Discoloration, bleaching and death can occur within 1 to 2 d of exposure to high levels of COS, although partial mortality of colonies may be somewhat delayed. The skeleton of dead corals is white and remains so for weeks before being overgrown by algae. Discolored, bleached and dead coral colonies are readily visible to field researchers, often from a distance (authors' pers. obs.). Entire colonies can display such signs when treated with COS baths in the laboratory. In contrast, any such signs in field collections are limited to the small parts of colonies that are directly exposed to COS during targeted collecting of individual fish.

While the 3 experimental studies clearly demonstrate that large doses of COS at high concentrations and delivered at close range have strong adverse effects on corals, the concentrations and dosages that produced these results are rarely used in research collecting of reef fishes. While a large amount (100 ml) of

strong COS applied to a coral colony in the field can produce lasting damage (partial mortality), the area of such damage is very small (~5 cm in diameter), and limited to the point of application. Observations by researchers indicate that corals occasionally display minor signs of stress (localized, temporary discoloration) in response to the small doses of high-strength COS typically used when collecting individual, unconfined fish in the field. However, single applications of small amounts (10 ml) of 100 ppt ALCOS into the center of finely branched coral colonies and even repeated doses of large amounts (120 ml) of 70 ppt AQCOS may produce no obvious signs of stress in corals.

The rapidity with which high-strength COS normally dissipates in open water reduces the potential for adverse impacts on corals during collections of individual fish. Repeated exposure of small parts of coral colonies to COS occurs most often in experimental studies (5 of those analyzed here) that involve the capture of inquiline fishes that associate closely with, and depend strongly on live corals. However, there was little evidence of adverse reactions by corals in these studies and the occasional reactions were slight and temporary. Researchers have a strong vested interest in minimizing COS use to avoid any coral damage that would jeopardize the integrity of experiments with such inquiline fishes.

In confined-assemblage collections under porous tents in open water, corals can be exposed to COS for between 1 and ~15 min. Initial potential concentrations of COS (which would rapidly decline due to leakage and dispersion) are ~1 to 5% of those used when catching individual fish using squirt bottles. Exposures to similar concentrations of COS for similar durations in aquaria (with no increasing dilution, unlike the field situation) produce little or no obvious coral damage.

COS concentrations used in tide-pool collections are very low, and are lower than those used under tents in open water. However, exposure times in tide pools, where there is no immediate onset of a decline in concentration, can be for several hours. Effects of such treatment regimes on corals remain unknown.

When delivered in large quantities at close range, concentrated ALCOS produces a stronger adverse reaction from corals than AQCOS containing the same percentage of clove oil. Moreover, a large dose of ALCOS at close range produces a much stronger adverse reaction than a similar sized dose of high strength ethanol. Exposure of corals to laboratory baths of 5% ethanol in seawater for 60 min can produce less obvious stress than weaker baths of ALCOS. These results indicate that the dissolution of clove oil in alcohol renders the oil more toxic to corals. However, even if ALCOS is more toxic than AQCOS containing the same concentration of clove oil, there may be no net advantage to using AQCOS if its immiscibility with water means that larger quantities are needed per fish.

The total amount of substratum that was exposed to COS during any study involving collection of fishes was typically small: an average of 9 m^2 (range 0 to 17 m^2) of live coral in each of 7 studies in which COS was used to catch unconfined fish; 3 to 5 m^3 of pool in each of 3 tide-pool studies; and 33.3 m^2 (range 10.1 to 86.5 m^2) of various substrata in 5 confined-aggregation studies in open water. Because other substrata as well as corals were sampled in both types of confined-aggregation collections, the actual areas of live coral that were exposed to COS could have been much smaller than these values.

Real-world experiments on the effects of clove oil are essential

With the aim of minimizing possible adverse responses of corals and other organisms to COS usage, field experiments should be made to establish (1) minimum concentrations of clove oil (and alcohol) for effective collecting of live unconfined fishes in the field; (2) minimum dosages (concentration × duration of exposure) for effective confined-aggregation collections in open water; and (3) minimum dosages (concentration × duration) for effective collections in tide pools. The only situation in which aquarium experiments might be substituted would be in determining dosages for tide-pool collections (cf. Griffiths 2000). Concentrations and dosages that are used to collect live unconfined fish will likely vary, with small, slow species that are strongly attached to small areas requiring less and weaker COS than larger, more agile and mobile species. Usage of these minimum concentrations and dosages should be adopted as 'best practice', based on knowledge of the behavioral characteristics and relative mobility of the target species and the objectives of the research.

Existing experimental studies do not provide a sufficient understanding of the effects of field usage of COS on live corals, mainly because they did not accurately simulate the range of modes of actual field usage, and because they were of short duration. The primary focus of future assessments of damage potential should be on field experiments. These should examine the effects of ALCOS and AQCOS on a variety of indicators of coral stress (discoloration, bleaching, and partial and/or complete mortality), as well as growth and reproduction. As there can be substantial seasonal fluctuations in the densities of zooxanthellae and pigments in hard corals that are not visibly dis-

cernible by divers (Fitt et al. 2000), assessments of stress and recovery could also include direct measurements of these densities and of changes in photosynthetic capability (cf. Frisch et al. 2007). Assessments of stress effects must be done at ecologically significant time scales, e.g. seasonal or annual, to gauge the extent of long-term stress and the capacity of corals to recover from any negative effects of COS exposure. Field tests are necessary because laboratory conditions may impose hidden stresses that are not evident in control corals but strengthen adverse reactions of corals exposed to COS. Experiments should primarily use entire adult colonies rather than colony fragments, because (1) unexposed parts of an entire colony may enhance recovery from negative effects that are experienced by a small proportion of the colony, and (2) fragments are unlikely to represent useable habitat for fishes. Any use of fragments must allow adequate acclimation time before treatments are administered. The point at which growth has visibly resumed and is readily discernible (~15 to 20 d, cf. Jones 1997) may be appropriate. Species of corals that harbor inquiline fishes and are most likely to be collected with COS are most appropriate for tests of adverse COS effects, particularly repetitive treatments. As responses to stresses such as elevated water temperatures that produce bleaching vary among coral species (e.g. Huerkamp et al. 2001), variation among coral species in responses to COS applications should be expected. Separate tests need to be made using the 3 modes of COS application used in field collections: (a) highly targeted collecting of live, unconfined fish using brief exposures to small amounts of high-strength COS; more sustained exposure to low strength COS used for confined-assemblage collections, including (b) nondiluting baths in tide pools, and (c) diluting baths under tents in open water.

Any laboratory experiments to assess the effects of COS on coral physiology must accurately simulate one or more of the 3 modes of COS use noted in the previous paragraph. Results from one mode of use cannot be assumed to be valid for another.

Is scientific collecting a significant problem for management?

As increasing attention by managers becomes focused on research collecting on coral reefs, it becomes essential to ask whether the adverse effects of COS use in field studies of coral reef fishes represent a biologically significant problem for individual corals exposed to COS, and for the health and maintenance of coral populations and coral reefs.

The 3 experimental studies on the effects of COS on corals implied that there is a potentially significant problem with such COS usage because it has come into common use among reef-fish biologists in many parts of the world. Frisch et al. (2007, p. 102), for example, stated that '… there may be hundreds to thousands of clove-oil users in Australia alone'. There is no evidence from the number of publications produced over the last decade of such a level of activity. There simply are not that many coral-reef fish biologists (scientists and graduate students) who are currently active in Australia, and most of them are not using COS to collect reef fishes.

We were able to obtain information on 15 studies (that produced 31 publications) conducted since 2000 that involved the use of COS to collect coral reef fishes, plus 2 that collected reef fishes in other habitats (an average of <2 publications yr^{-1}). Searches of Biological Abstracts, Zoological Record, the Web of Science and Google Scholar using key words such as 'clove oil' and 'fish', identified less than a quarter of these publications. This number is an underestimate of the actual number of studies because some studies might not have recorded their use of COS and others that involve COS usage might not have been published. Thus, we assume that there might be 50 to 100 studies yr^{-1} worldwide that involve the use of COS to collect tropical reef fishes, which would mean that publications enable detection of only ~2 to 3% of the actual usage.

The potential effects of such a level of research activity (~50 to 100 studies yr^{-1}) on corals can be placed in perspective as follows: first, the total area of live coral tissue subjected to COS exposure during any single study would be very small, i.e. <10 m^2 on average, for a total of ~500 to 1000 m^2 yr^{-1} (~40 to 80% of the surface area of an Olympic swimming pool) worldwide. This amount stands in stark contrast to the 285 000 km^2 occupied by structural coral reefs worldwide (Spalding et al. 2001). Further, large areas of shallow tropical habitats that lack coral reefs support dispersed coral growths. For example, while there are only ~25 km^2 of structural coral reefs in the tropical eastern Pacific, Glynn & Ault (2000) noted that the region includes ~15 000 km^2 of shoreline habitats that are capable of supporting reef development, and where scattered coral growths commonly occur (D. R. Robertson pers. obs.). Although this region may be an extreme example, it clearly demonstrates that corals are common organisms not only on structural coral reefs but also in other habitats. Even assuming an average coral cover of only 10% on a structural coral reef, sampling of reef fishes with COS would expose <<0.000005% of the world's coral populations to COS during any year.

Second, there is simply no comparison between the scales of any brief effects produced by COS-based

field research and that of major damage produced either by acute natural events such as floods, storms, hurricanes, volcanic eruptions, and tsunamis or by large-scale, long-term and increasing negative human impacts brought about by global warming, pollution, coastal development, anchor damage, recreational SCUBA diving, and overfishing. Such acute natural events affect corals and coral reefs on the scale of 10s to 1000s of km^2, while long-term, human-induced stresses affect corals and coral reefs virtually throughout the entire tropics.

Finally, the scale of research collecting of coral reef fishes needs to be placed in perspective relative to the scale of other human extractive usage of tropical reef fishes. This includes commercial and recreational fishing, and the collection of live fishes for both the live food-fish and ornamental aquarium-fish trades. Methodologically, scientific collecting of live reef-fishes resembles commercial live-fish collecting. However, such commercial fishing involves many thousands of collectors and millions of individual fish (Wood 2001, Sadovy et al. 2003), and individual commercial collectors are much more continuously active throughout the year than most researchers. Hence, the scale of this commercial activity and its attendant environmental effects are vastly greater than those of scientific collecting.

All this readily available information demonstrates that any negative effects of tiny, brief research collections using COS are inconsequential relative to the capacity of coral populations and coral reefs to recover from temporary environmental stress, especially when compared to the effects of large-scale natural and human-induced events on coral populations. However, in defending research collecting and the use of clove oil for collecting coral reef fishes, we emphatically are not advocating that the environmental effects of such an activity be ignored, or that such collecting be allowed anywhere at any time. Proposals for such activities should be assessed individually based on their merits, and particularly sensitive sites obviously should not be open to collecting. Rather, we urge managers to recognize and take into account the minuscule environmental effects of individual research projects that involve collecting of coral reef fishes, and to allow limited levels of this activity when it has sound scientific or management goals.

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AS WE SEE IT

Use of clove oil in collecting coral reef fishes for research

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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 nonrepetitive, 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).

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