TECHNICAL NOTE

In Situ Tagging and Tracking of Coral Reef Fishes from the *Aquarius* Undersea Laboratory

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ABSTRACT

We surgically implanted coded-acoustic transmitters in a total of 46 coral reef fish during a saturation mission to the *Aquarius* Undersea Laboratory in August 2002. *Aquarius* is located within the Conch Reef Research Only Area, a no-take marine reserve in the northern Florida Keys National Marine Sanctuary. Over the course of 10 days, with daily bottom times of 7 hrs, saturation diving operations allowed us to collect, surgically tag, release, and subsequently track fishes entirely *in situ*. Fish were collected using baited traps deployed adjacent to the reef as well as nets manipulated on the bottom by divers. Surgical implantation of acoustic transmitters was conducted at a mobile surgical station that was moved to different sites across the reef. Each fish was revived from anesthetic and released as divers swam the fish about the reef. Short-term tracking of tagged fish was conducted by saturation divers, while long-term fish movement was recorded by a series of acoustic receivers deployed on the seafloor. Though not designed as an explicit comparison with surface tagging operations, the benefits of working entirely *in situ* were apparent.

INTRODUCTION

The use of acoustic telemetry to track the movements of marine fishes is now a commonly employed method (see other papers this issue), producing data on fish home ranges (Zeller, 1997; Bolden, 2001; Simpfendorfer et al., 2002), habitat-specific movement (Lindholm and Auster, 2003; Lowe et al., 2003; Cote et al., 2004), and movement relative to the boundaries of marine protected areas, or MPAs (Zeller and Russ, 1998; Meyer et al., 2000; O'Dor et al., 2001; Starr et al., 2001; Lowe et al., 2003). While the specific approaches to the use of acoustic telemetry vary widely depending on the species targeted for study and the habitat in which the targeted species occurs, all projects share four common elements: 1) the collection of fishes, 2) the tagging of fishes, 3) the release of tagged fishes, and 4) the tracking of tagged fishes. Also common to most studies is the conduct of each of these elements from the surface, where the extraction of fishes from their natural environment can create difficulties.

Fishes are often collected from the surface via baited traps, long-lines, trolling and traditional angling. Fish brought to the surface for tagging can experience barotrauma, or pressure-related stress. This is particularly true with deepwater fishes that have air bladders (Starr et al., 2000). Thermal shock can also result from bringing a fish to the surface and then aboard a surface vessel (Kelsch and Shields, 1996). Each of these stressors can kill a fish outright or have sub-lethal effects that increase the fishes' vulnerability to other stressors and may preclude a fish from being tagged.

Telemetry projects on fishes typically involve either the attachment of an acoustic transmitter externally (e.g., Bradbury et al., 1995; Lindholm and Auster, 2003; Cartamil and Lowe, 2004), intragastric insertion of the transmitter down the pharynx into the stomach (Bridger and Booth, 2003), or surgical implantation of the transmitter inside the peritoneal cavity of a fish (e.g., Zeller, 1997; Starr et al., 2000; Bolden, 2001; papers in this volume). Where surgical implantation is used, incisions can be closed with sutures (e.g., Thoreau and Baras, 1997), surgical staples (e.g., Mortensen, 1990) or an adhesive (e.g., Nemetz and MacMillan, 1988). Implantation is normally conducted on a padded, v-shaped surgical board (Winter, 1996) and may involve fresh flowing seawater over a fish's gills, or covering the fish with a damp towel. Each approach involves a series of trade-offs that will vary depending on the species selected for tagging, though most approaches involve exposure of the fish to air and sunlight for some period of time during surgery.

Following some period of observation in a live-well on board a research vessel, tagged fishes are either released directly over the side of the boat or are lowered to the seafloor in some form of a release device (e.g., Starr et al., 2000; Lindholm and Auster, 2003). Though the use of a release device can increase researcher confidence that the fish has indeed returned to the seafloor or at the depth from which it was collected initially, both forms of release involve a high measure of uncertainty. The fate of these fish post-release is difficult to determine, i.e., did the transmitter malfunction, was the tagged fish consumed by a predator, is the fish dead and lying on the seafloor? This problem is lessened when data are collected indicating the tagged fish is moving. However, where the post-release data are limited or non-existent, it is often impossible to know the fate of the fish without *in situ* observation.

Tracking of tagged fishes is often conducted manually from the surface (e.g., Matthews, 1992; Zeller, 1997; Cartamil and Lowe, 2004), recorded by acoustic receivers deployed on seafloor (e.g., Arendt et al., 2001; Starr et al., 2001; Lindholm and Auster, 2003), or a combination of the two approaches (e.g., Simpfendorfer et al., 2002; Lowe et al., 2003; Parsons et al., 2003). The approach used to study the movement of tagged fishes will depend heavily on the species tagged, the region in which it was tagged, and the objectives of the study.

Obviously, the success of acoustic telemetry approaches to tracking marine fishes is contingent on tagging fish in a manner that does not negatively affect their behavior and physiology (Smolowitz and Wiley, 1998; Bridger and Booth, 2003). Thus any approach that minimizes the potential stressors at each step of the process is preferable. In this paper we describe a study using acoustic telemetry to track movements of coral reef fishes in which all aspects of the project were conducted *in situ* from the *Aquarius* Undersea Laboratory (Figure 1).

Aquarius is currently located within the Conch Reef Research Only Area, a no-take reserve designated by the Florida Keys National Marine Sanctuary. It provided a platform for a 10-day saturation diving mission in August 2002. A total of 70 hrs of bottom time (~ 7 hr day^{-1}) provided saturation divers with sufficient time to tag and subsequently observe 46 fish (Table 1), including black grouper (*Mycteroperca bonaci*), yellowtail snapper (*Ocyurus chrysurus*), princess parrotfish (*Scarus taeniopterus*), blue parrotfish (*Scarus coeruleus*), and hogfish (*Lachnolaimus maximus*). We describe some of the lessons learned during the mission.

Fish Collection

Collection of fishes *in situ* provided us a high degree of selectivity in the fish we chose to tag. The majority of fishes were caught in baited traps (1.5 m^2) deployed at two locations on sand flats immediately adjacent to Conch Reef during daylight hours (Figure 2). Both locations were sited within 300 m of *Aquarius* to ensure easy access to saturation divers. Each trap was visited by saturation divers at the beginning of a dive, with subsequent visits depending upon the number and species of fishes caught by the trap. In cases in which multiple fishes within a trap were selected for tagging, the opening to the trap was blocked and each fish was tagged sequentially until the trap was empty. The trap would then be baited again and re-deployed.

TABLE 1

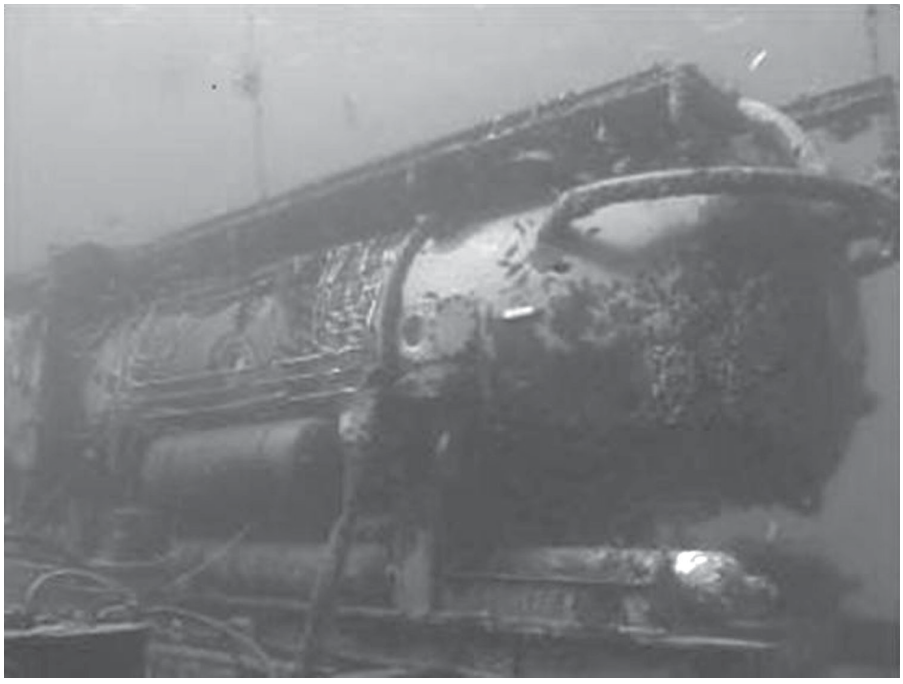
List of coral reef fish species tagged at Conch Reef during the August 2002 *Aquarius* mission.

Species	Individuals Tagged
Yellowtail snapper	14
Mangrove snapper	2*
Black grouper	1 (2*)
Scamp grouper	1
Hogfish	10
Blue parrotfish	8
Princess parrotfish	
Initial Phase	4
Terminal Phase	8

*These fish were tagged from the surface immediately prior to the start of the mission.

FIGURE 1

The *Aquarius* undersea laboratory (bow and starboard view) at Conch Reef in the northern Florida Keys National Marine Sanctuary. Photo credit: James Lindholm

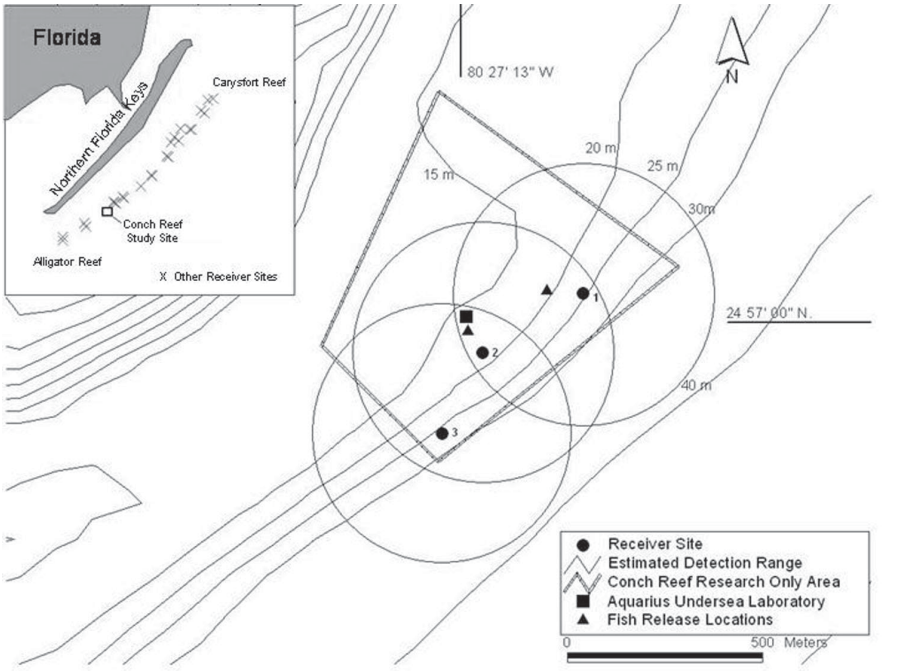


Yellowtail snapper, blue and princess parrotfish, as well as black grouper were each caught in traps during the saturation mission. The multiple yellowtail snapper captured in traps appeared to be attracted to the bait (punctured cans of cat food), while the single black grouper caught in a trap appeared to be attracted more to the variety of fishes contained within the trap. Both species of parrotfishes appeared to enter the traps out of curiosity, often following conspecifics into the trap. Other species that were captured (but not tagged) included chub (*Kyphosus* spp.), French grunt (*Haemulon flavolineatum*), reef butterflyfish (*Chaetodon sedentarius*), gray angelfish (*Pomacanthus arcuatus*), blue tang (*Acanthurus coeruleus*), doctorfish (*Acanthurus chirurgus*), and green moray (*Gymnothorax funebris*).

Our ability to capture fish in greater numbers may have been reduced by the presence of large predators immediately adjacent

FIGURE 2

Map of VR2 acoustic receiver array at Conch Reef, including the estimated range of detection for each receiver, the boundary of the Conch Reef Research Only Area, the location of the *Aquarius* undersea laboratory, and the locations where fish were collected and released. Five meter isobaths are provided. The complete receiver array (inset) is shown extending from Alligator Reef to Carysfort Reef in the northern Florida Keys.



to the traps. A nurse shark (*Ginglymostoma cirratum*) was commonly observed swimming near one of the traps, and on one occasion was found resting on top of a trap. Though there were no observed acts of predation within the trap, on two occasions a green moray was found in the trap stationed adjacent to *Aquarius*. In each case the trap was otherwise devoid of fish, though no obvious distensions of the eel's stomach were observed so it was not clear whether trapped fish had been consumed. During the November 2001 *Aquarius* mission, a green moray when disturbed egested a black grouper similar to one that had previously been seen in the trap (Lindholm et al., unpublished observations).

In addition to the selective tagging of fishes from the traps, the extensive bottom times provided by saturation diving also allowed us to selectively target individual fish for capture using alternative approaches. A standard beach seine net was used to corral fish that were foraging on the sand flat adjacent to *Aquarius*. In each case, one end of the net was anchored to the seafloor while the opposing end was handled by a diver. A second diver would gradually herd fish toward the net as the other diver closed the net (as per Eristhee et al., 2001). Using this approach, a total of 5 hogfish (which generally avoided the traps) and a blue parrotfish were captured. Though time consuming, the use of the net allowed us to select particular fish for capture and tagging with some success. An additional 3 hogfish and a scamp grouper were herded directly into a diver's mesh bag by one of the *Aquarius* technicians while conducting maintenance on the lab's external surfaces. The hogfish were attracted to the fouling being chipped off the *Aquarius* support columns by the technician.

The selectivity provided by each of these three approaches (trap, net, bag) would either not have been possible with most surface tagging operations, or would only be possible to a limited extent as surface divers would be constrained to shorter bottom times. By effectively "pre-screening" the fishes prior to surgery (Starr et al., 2000), we were able minimize the collection of non-targeted species, a major consideration when working within a fully-protected marine reserve. While collection of non-targeted fishes was common with the traps, all were released shortly after capture and with the exception of the possible consumption by the green morays, no by-catch mortality was observed.

Fish Tagging

The conduct of surgical tag insertion *in situ* is not new (Starr et al., 2000), but is less common than tagging from the surface. In the present study all fishes were surgically tagged with acoustic transmitters at the same location and depth where they were collected. Protocols for surgery were developed during the November 2001 *Aquarius* mission (Stone, 2003). Results from previous tagging efforts at Conch Reef indicated that the number of signal detections for yellowtail snapper and black grouper was significantly higher for those fish with intra-peritoneal transmitters when compared to externally attached transmitters (Lindholm et al., unpublished observations). We hypothesized that the externally attached transmitters, particularly for yellowtail snapper, served to attract predators and disrupt swimming ability.

Once a captured fish was selected for surgical implantation of a transmitter, it was isolated and removed from the trap using a diver's mesh bag. While in the mesh bag, each fish was anesthetized using seawater-buffered MS-222 solution (100 mg l^{-1}; Summerfelt and Smith, 1990) contained in a 120 ml syringe. Administering anesthetic at the surface often involves submerging a fish in an anesthetic bath until complete stupor is achieved. This approach has proven successful, but may expose fish to higher dosages of anesthetic than are necessary. Further, the effective and toxic doses of MS-222 are similar, particularly in warmer waters (Bridger and Booth, 2003). Using the syringe, the anesthetic was introduced directly into the mouth of the fish while still in the mesh bag. Once signs of stupor were evident, fish were removed from the mesh bag and additional anesthetic was provided if necessary. In this way each fish was exposed only to the minimum amount of an-

esthetic necessary to attain complete stupor. Stage IV anesthesia was achieved in 1-3 minutes depending on species and fish size.

Following anesthesia, each fish was removed from the mesh bag and placed in a mesh sling designed to support the fish during surgery (Figure 3). The sling was supported by a PVC frame and was easily transported by divers to different locations on the reef. A 25 mm incision was made just off the ventral line on the left side of the fish, forward of the vent. Each fish was tagged with a V8SC-1H (69 kHz) coded-acoustic transmitter (VEMCO, Ltd., Shad Bay, Nova Scotia). Each transmitter, measuring 3.3 cm in length and weighing 3.5 gm in seawater, randomly produced a unique ID code at intervals between 60 –180 seconds. Each transmitter was coated with triple-antibiotic ointment and inserted into the peritoneal cavity. The incision was closed using 1 to 3 black monofilament sutures (5-0 Ethilon) and coated with triple-antibiotic ointment. Total length (TL) was measured for each fish and an external flag tag was inserted into the musculature at the base of the first dorsal fin.

The release of tagged fish *in situ* following surgery provided considerable advantages over a release from the surface. A diver swam each tagged fish along the seafloor to flush its gills with "fresh" seawater until it completely revived. Each fish required 2-5 minutes to revive completely depending on the size and species. Once fully revived, each fish was released on the seafloor and was observed for 5 minutes, or until the fish swam out of visual range. All 46 tagged fishes were observed to recover from the surgical procedure and resume normal swimming behavior following release. The single mortality we observed occurred when a tagged yellowtail snapper was consumed by large black grouper shortly after release by a diver. The strike occurred in the water column as the yellowtail snapper swam toward a large aggregation of conspecifics.

Observation and Tracking of Tagged Fishes

Another distinct advantage of conducting *in situ* operations was our ability to observe and evaluate the condition of most tagged fishes following release. A diver-held VUR96 receiver (VEMCO Ltd., Shad Bay, Nova Scotia) was used to track a subset of all tagged fishes. In addition to the coded V8SC-1H transmitters, a total of 8 fishes were also tagged with V8SC continuous transmitters, including 2 black grouper, 4 blue parrotfish, and 2 mangrove snapper (*Lutjanus griseus*). The 2 black grouper and 2 mangrove snapper were tagged from the surface immediately prior to the beginning of the mission. These transmitters were similarly dimensioned to the coded transmitters, but transmitted a signal each second. Using the diver-held receiver, each of the 8 fish was located over the course of the 10-day mission on multiple occasions. Though saturation diving rules limited the distance divers were permitted to venture away from *Aquarius*, fish located using the diver-held receiver were observed for periods of up to 15 minutes at a time or until the fish swam out of visual range.

Additional tagged fishes were identified by their external tags and were observed for comparable periods of time (Figure 4). Though the tag ID numbers were not possible to read without re-capturing the fish, the combination of fish size, location on the reef, and unique markings or scars allowed us to identify particular fish with high confidence. Each of the 10 tagged hogfish were observed in this manner, as were each of the 8 blue parrotfish, several of the princess parrotfish (both initial and terminal phase fish), and two of the 13 tagged yellowtail snapper. In each case, the primary observation was to confirm the fishes were alive and swimming freely. Additional observations included post-tagging feeding behavior and microhabitat utilization. The behavior of tagged fishes was compared to untagged control fish from each species to determine whether tagging had altered fish behavior (Lindholm et al., unpublished observations).

Long-term patterns in the movement of tagged fishes (up to 11 months) were recorded by three, omni-directional, single-channel (69 kHz) VR2 acoustic receivers (VEMCO, Ltd., Shad Bay, Nova Scotia) deployed at Conch Reef (Figure 2) at 25 m (Site 1), 20 m (Site 2), and 25 m (Site 3), respectively. Each VR2 receiver operated in continuous receiving mode, recording the presence/absence of tagged fish within a

FIGURE 3

Saturation divers conduct surgical implantation of an acoustic transmitter in a coral reef fish using a portable PVC surgical station. Photo credit: Rick Riera-Gomez/RSMAS

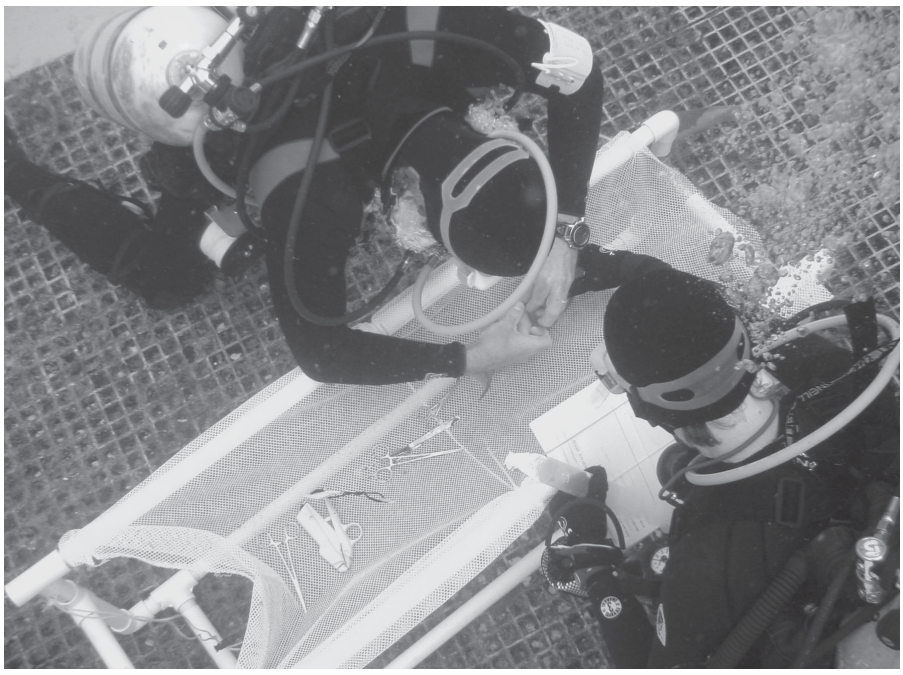
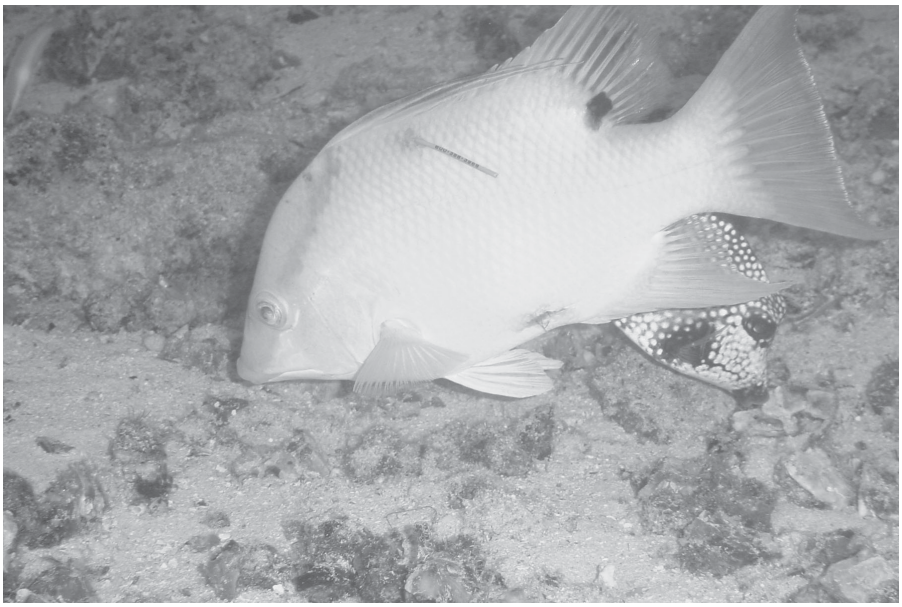


FIGURE 4

A tagged hogfish forages adjacent to *Aquarius* one day following surgical implantation of an acoustic transmitter. The surgical incision is visible just off the ventral line, forward of the anal fin, while the external flag tag is clearly visible at the base of the dorsal fin. Photo credit: Rick Riera-Gomez/RSMAS



range of detection of approximately 300 m (based on preliminary testing). Receivers were placed by divers from the surface immediately prior to the *Aquarius* mission. They were mounted on bars drilled into the reef that maintained the receiver heads oriented toward the surface at a height of 1 m above the seafloor.

Placement of the receivers inside the Conch Reef Research Only Area provided protection from disturbance by recreational diving and fishing activity, both of which are excluded from the area surrounding *Aquarius*. An additional 24 VR2 receivers were placed along the reef tract from Alligator Reef in the south to Carysfort Reef in the north, encompassing 40 km (Figure 2 inset). Many of these receivers were also located within Sanctuary Preservation Areas (another type of no-take reserve within the Florida Keys National Marine Sanctuary). They were sited to capture any movement of tagged fishes away from Conch Reef.

Data were collected by the 3 VR2 receivers deployed at Conch Reef for 49 of the 50 tagged fish during the first month following release, with data for selected individual fish collected for up to 11 months. No data were collected on the yellowtail consumed shortly after release. Further, no data were collected at receivers to the north or south of Conch Reef (Lindholm et al., unpublished information). All receivers were deployed from August 2002 through July 2003, three weeks following the last record of a tagged fish.

Conclusions

The conduct of a fish tagging project entirely *in situ* from *Aquarius* proved highly successful. Though we did not design this study with an explicit surface tagging component for a direct comparison, the experience was instructive. Most importantly, tagged fishes never left the depths at which they occurred naturally. The capture and surgical tagging of coral reef fishes without bringing them to the surface minimized stress on the selected fishes, and minimized the capture of non-targeted species. The sub-surface release of fish by divers and the subsequent observation of post-surgery fish behavior over the course of 10 days provided further evidence of the benefits of working *in situ.* This provided a level of confidence in the resulting movement data that is not often possible with operations conducted from the surface.

Obviously, saturation diving is not possible under all circumstances. *Aquarius* is currently the only undersea laboratory in the world dedicated to scientific research (Miller and Cooper, 2001). However, though our ability to conduct the project entirely *in situ* derives largely from the extensive bottom time provided by saturation diving, saturation need not occur to successfully conduct operations underwater. For instance, Starr et al. (2000) developed an approach for tagging deepwater rockfishes using standard SCUBA to tag fish at a surgical station deployed 20 m below a surface vessel. Fish caught on hook-and-line were reeled up to divers waiting at the station, and were returned to the seafloor post-tagging in a release device without ever reaching the surface.

Ultimately, the object of any acoustic telemetry project is to produce data in which the investigators have high confidence that the recorded patterns in movement are indeed reflective of living fish behaving similarly to untagged conspecifics in the same area. To the extent that any component of a telemetry project can be conducted *in situ*, our experience suggests it will be worth the effort.

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