PAPER

In situ Tagging of Nassau Grouper *Epinephelus striatus* Using Closed-Circuit Rebreathers at a Spawning Aggregation in Puerto Rico

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ABSTRACT

Acoustic telemetry is a widely used technique employed to better understand fish movement patterns across seascapes. Traditionally, surgical acoustic transmitter implantation is conducted at the surface, resulting in a high degree of uncertainty as to the post-release survival of the fish and the validity of the results attained from these experiments. Few studies have conducted *in situ* tagging, where the capture, tagging, and release are completed entirely at the depth in which the fish occurs naturally. Through the use of closed-circuit rebreather (CCR) technology, this study outlines the first known practical application of the methodology performed at mesophotic depths. In six dives conducted at depths between 40 and 50 m, a total of 10 Nassau grouper were tagged at a spawning aggregation off the west coast of Puerto Rico. The total time (time divers arrived at the trap to time of release) for each procedure was approximately 12 min, after which all fish were released and observed without indication of stress or physiological impairment. Short-term tracking of tagged fish revealed a 100% post-surgery survival rate with maximum detection of 347 days post-surgery. Survival rates of this nature have not been quantified or reported from other tagging studies, allowing the researchers to conclude that this methodology, coupled with the efficiency provided by CCR at these depths, enhanced survivorship and bias for studies utilizing acoustic telemetry.

Keywords: acoustic telemetry, *in situ* tagging, Nassau grouper, closed-circuit rebreathers, mesophotic

Introduction

Over the past decade the use of acoustic telemetry via surgically implanted acoustic transmitters to track fish movements has become increasingly popular (Starr et al., 2000; Bolden, 2001; Semmens et al., 2005; Lindholm et al., 2005; Starr et al., 2007; Semmens et al., 2010; Cook et al., 2011; Welsh et al., 2012; Farmer et al., 2013; Pittman et al., 2014). Extended transmitter battery life and high storage capacity receivers have enabled researchers to track the movement of individuals for periods of months to years, providing input about fish home range, site fidelity, and movement relative to habitat features or the boundaries of marine protected areas (MPAs) (Bolden, 2001; Lindholm et al., 2005; Appeldoorn et al., 2009; Cooke et al., 2011; Farmer & Ault, 2011; Welsh et al., 2012). Specifically, the movement patterns of economically and ecologically important species have been of significant interest to marine resource managers, and results have proven essential for the proper implementation of regulations to protect these species (Appeldoorn, 1997; Cooke et al., 2011). The validity of results is highly dependent on the method in which transmitters are deployed, as this relates directly to the primary assumption underlying tagging studies, i.e., that tagged individuals behave in a way that resembles that of their untagged conspecifics. Thus, tagging procedures should be designed such that the surgical procedure does not impair the behavior, vulnerability, and physiology of the fish (Bridger & Booth, 2003; Lindholm et al., 2005; Cooke et al., 2011).

Traditionally, surgical procedures have been implemented on the surface with fish capture conducted using fish traps, traditional angling, nets, and long lines (Bolden, 2001; Semmens et al., 2005; Lindholm et al., 2005; Starr et al., 2007; Semmens et al., 2010; Feeley et al., 2012). These procedures require that individuals caught at depth be transported to the surface, introducing a number of potentially fatal stressors and sublethal effects

that significantly reduce the survival rate of the fish and ultimately compromise the outcome of the tagging study (Bridger & Booth, 2003; Lindholm et al., 2005). Barotrauma—pressure-related stress caused by the overexpansion and possible rupture of the swim bladder—is the most detrimental effect to fishes retrieved from relatively deeper depths (Parrish & Moffitt, 1992; Starr et al., 2000; Bartholomew & Bohnsack, 2005; Rummer & Bennet, 2006; Campbell et al., 2009; Sumpton et al., 2010). A study conducted by Wilson and Burns (1996) found that survival rates for both red grouper (*Epinephelus morio*) and scamp grouper (*Mycteroperca phenax*) decreased to <33% with fish captured at depths greater than 44 m. Other lethal and sublethal stressors associated with surface tagging include thermal shock, physical trauma, predation, physiological imbalance, and prolonged exposure to sunlight and air (Lindholm et al., 2005; Campbell et al., 2009). A solution to these risks include methodologies utilizing true *in situ* tagging where the capture, tagging, and release are completed entirely at the depth in which the fish occurs naturally (Lindholm et al., 2005; Feeley et al., 2012).

In situ tagging is an unconventional procedure compared to traditional fish tagging. Starr et al. (2000) first conducted *in situ* tagging procedures on deep-water rockfish. However, this method involved reeling the fish to a manageable depth for normal diving operations, subjecting the fish to barotrauma and other physiological stressors. Lindholm et al. (2005) conducted *in situ* tagging via saturation diving missions at the *Aquarius* Undersea Laboratory. This method, although highly successful, is impractical for scientific diving conducted at remote locations, as it requires an underwater living facility. Similarly, both of these studies completed tagging at a depth of 20 m. However, to date there has yet to be a practical method developed for true *in situ* tagging for fish that occur at deeper depths.

Over the past several decades, the use of closed-circuit rebreather (CCR) technology has become increasingly available to the scientific diving community (Pyle, 2000; Lindfield et al., 2014). Closed-circuit rebreathers offer several advantages over traditional open circuit (OC) diving, including higher gas efficiency, lower operational costs, shortened decompression obligations, and near silent operations (Pyle, 1999; Bozanic, 2002; Parrish & Pyle, 2002; Butler, 2004; Tetlow & Jenkins, 2005; Shreeves & Richardson, 2006; Sieber & Pyle, 2010; Lindfield et al., 2014). Closed-circuit rebreather technology differs from OC technology in that the divers exhaled breath is no longer expelled into the surrounding environment but rather is recirculated, chemically scrubbed of carbon dioxide, replenished with oxygen, and returned to the diver (Pyle, 2000; Bozanic, 2002; Shreeves & Richardson, 2006). Rebreathers deliver a dynamic breathing mixture by maintaining a preset, optimal oxygen partial pressure at all depths, thus significantly reducing the diver's decompression obligation, while increasing bottom time duration and depth capabilities (Pyle, 1999; Shreeves & Richardson, 2006, Sieber & Pyle, 2010). Additionally, bubble and noise-free operation enhances the diver's ability to both approach fish and observe behavior unaffected by diver presence (Lobel, 2001; Cole et al., 2007, Lindfield et al., 2014). The use of CCR technology has recently opened environments at mesophotic depths (30–150+ m, Hinderstein et al., 2010) to more extensive and rigorous study (e.g., Pyle, 2000; Sherman et al., 2010; Garcia-Sais, 2010; Bejarano et al., 2014), and with this comes the need to investigate ecological processes, with the issue of connectivity and the application of acoustic tagging being at the forefront.

The purpose of this study was to develop and implement the methodologies for conducting true *in situ* acoustic transmitter implantation using CCR technology. Motivation for this study was to gather information on the movements of the Nassau grouper (*Epinephelus striatus*) relative to its habitat and reproduction. This species is known to form large spawning aggregations, yet has been overfished throughout its range (Aguilar-Perera, 2006; Sadovy de Mitcheson et al., 2008; Schärer et al., 2012). It is currently considered threatened by the International Union for the Conservancy of Nature (IUCN), so quantification of movement patterns, especially relative to reproduction, would have high conservation value (Cornish & Eklund, 2003).

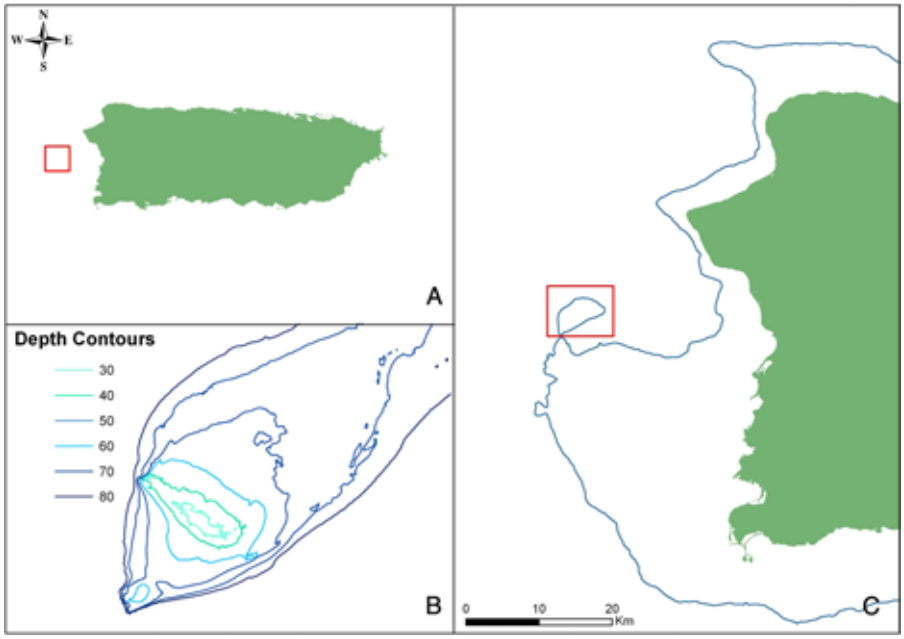
Materials and Methods

Site Description

All diving operations of this study were conducted at Bajo de Sico (BDS), a seamount located in the Mona Passage, 27 km off Puerto Rico's western coast (Figure 1). Reef bathymetry is characterized by a ridge of highly rugose rock promontories ranging in depths from 25 to 45 m, which rises from a mostly flat, gradually sloping shelf that extends to 100 m. Below this depth, the shelf ends in a vertical wall that reaches depths of 200–300 m to the southeast and over 1,000 m to the north. The dominant oceanographic features and its location within the Mona Passage

FIGURE 1

Bajo de Sico Bank (18°14′N, 67°26′W), Puerto Rico. (A) Study location 27 km off the west coast of Puerto Rico. (B) Depth contours depicting bathymetric features between 30 and 80 m. (C) Blue line indicated the 200-m depth contour. (Color version of figures are available online at: http://www.ingentaconnect.com/content/mts/mtsj/2015/00000049/00000001.)



make this area subject to periods of strong, persistent northerly currents. The area harbors highly diverse and taxonomically complex fish assemblages and is a known Nassau grouper spawning aggregation site (Garcia-Sias et al., 2007). All dives conducted for the purpose of tagging were completed during the days following the full moons of the winter months, to coincide with the grouper spawning aggregation period (Colin, 1992; Whaylen et al., 2006; Schärer et al., 2012).

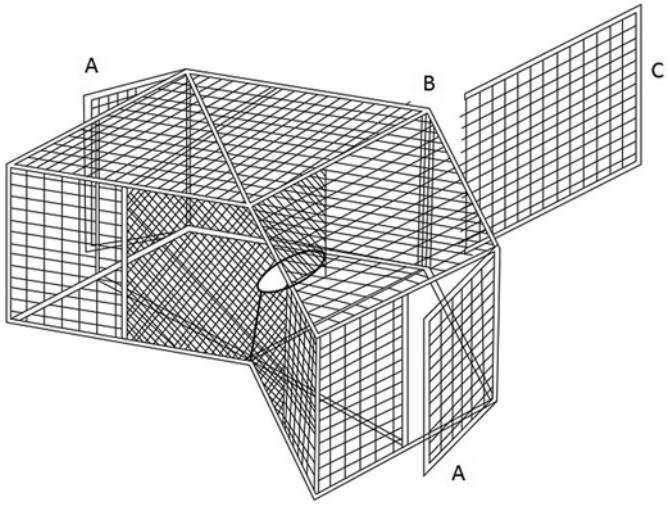
Fish Capture

In situ fish collection was conducted using Antillean arrowhead fish traps (dimensions: 1.2 m × 1.2 m × 50 cm). The traps were composed of a 3/8-inch rebar frame covered with 2.54 cm mesh PVC-coated chicken wire. Each trap contained two-side doors for fish removal and a thin vertical slot (approx. 2.3 × 50 cm), opposite the entry chute, through which a panel could be inserted to guide fish toward one of the doors (Figure 2). Trap design and divider implementation were developed based on earlier procedures utilized by the Florida Fish and Wildlife Conservation Commission (A. Acosta and P. Barbera, personal communication). The panel was constructed of 2.54-cm mesh PVC-coated chicken wire enclosed in a frame made of 1.27-cm PVC, which added structural support as well as prevented snagging of the wire mesh while the divider was slid into the trap.

A total of four traps were deployed at 40- to 50-m depths along the base of two sand-bottom channels. These locations were chosen prior to deployment based on their minimal live benthic cover and position relative to the dominant current regime. Trap locations were in close proximity to the spawning aggregation site, which potentially increased catch per unit effort and allowed for the traps to be observed by open circuit divers working in the area. The distance between trapping locations was such that all four traps could be serviced on a single CCR dive. Divers utilized the Inspiration™ (Ambient Pressure Diving®, Cornwall, United Kingdom) electronically controlled CCR (eCCR) units,

FIGURE 2

Diagram depicting Antillean arrowhead fish trap with specific modification for *in situ* extraction: (A) trap doors, (B) vertical slot, and (C) divider panel.



with either air (21% O_2) or Normoxic trimix (16/36/48 O_2/N/He) diluent depending on the maximum depth of the dive. Dive plans were generated to allow for a maximum bottom time of 50 min and a total bottom time of 110 min, with sufficient bailout gas carried by all divers in the event of a bailout scenario. Overall, bottom times varied according to the amount of work completed during each dive, but averaged 35 min yielding total bottom time of 80–90 min per dive.

Upon arrival to the study site, CCR divers would descend and bait each trap with a combination of squid and punctured cans of cat food. The trap doors were then closed, and traps were allowed to soak for a period of 4 h before they were checked again for the presence of target species. After the soak period, a second CCR dive was performed to conduct surgical tag insertion and/or re-bait the traps. If no target species were caught, all by-catch were released if present, and the traps were re-baited and allowed to soak overnight. The maximum allowable soak time for baited traps was 24 h. This time interval was chosen to reduce the stress and potential damage to any captured fish and was confirmed to be appropriate by local fishers. If tagging operations could not be performed within a 24-h period, all bait was removed, the doors were opened, and the traps were left on the seafloor.

Surgical Tagging Procedure

All tagging procedures were conducted using a team of two or three CCR divers, each having a specific set of tasks. The lead diver was responsible for surgical tagging operations, fish extraction, and assisting with initial fish restraint. The second diver was responsible for fish extraction and restraint during tagging operations. The third diver (optional) assisted with fish removal, surgical procedures, and potential large predator deterrence. Specific modifications were made so that the additional equipment needed for tagging operations was easily accessible to the divers. A surgical tool sleeve was constructed from neoprene wetsuit material, making all surgical utensils easily accessible on the surgeon's left forearm, and a 24-cm-long heavy duty, nylon zipper was installed vertically along the center of a large (60 × 76 cm) clamp-style, top entry, metal frame nylon mesh catch bag (modified from Lindholm et al., 2005), giving divers access to the fish without it having to be removed from the bag. A centimeter ruler was attached to a 1-m piece of PVC so that total length (TL) could be measured while the fish remained in the bag.

Once a fish was captured and selected for surgical tag implantation, all CCR divers would surround the trap and the divider panel slid into place, isolating the fish to one side of the trap (Figure 3). One to two CCR divers would position themselves in front of the trap door with the catch bag as another CCR diver gently guided the fish through the door and into the bag. Once in the bag, the fish was restrained in an upside down position with its eyes covered by the catch bag inducing a calm state. Anesthesia was not used because of (1) the reported negative effects of chemical anesthetics, (2) the prolonged time required for its administration and lengthy recovery times (U.S. FDA 2011; Akins et al., 2014; Nordgreen et al., 2014) were not feasible at depth, and (3) the catatonic state induced by inverting the fish ventral-side-up proved sufficient for safe handling. The zipper was positioned and opened around the pelvic fins to expose the incision site, approximately 3–4 cm posterior of the pelvic fin girdle (Figure 4). Using a scalpel, scales were removed from the incision site, and a 2.2-cm incision was made anterior to posterior on the ventral surface of the peritoneal cavity. Initially, the scales around the incision site were left intact in order to decrease the potential occurrence of tissue necrosis (Cooke et al., 2011). However, the presence of scales inhibited the incision closure by preventing the staples from puncturing the epithelial tissue; therefore, the decision was made to remove the scales prior to making the incision. Special care was taken so that

FIGURE 3

Photograph of CCR divers performing *in situ* extraction of Nassau grouper: (A) trap and divider panel pre-insertion and (B) trap and divider panel post-insertion.

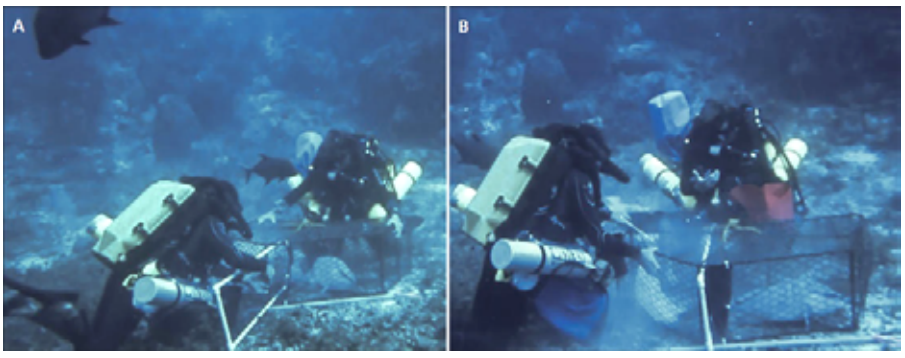
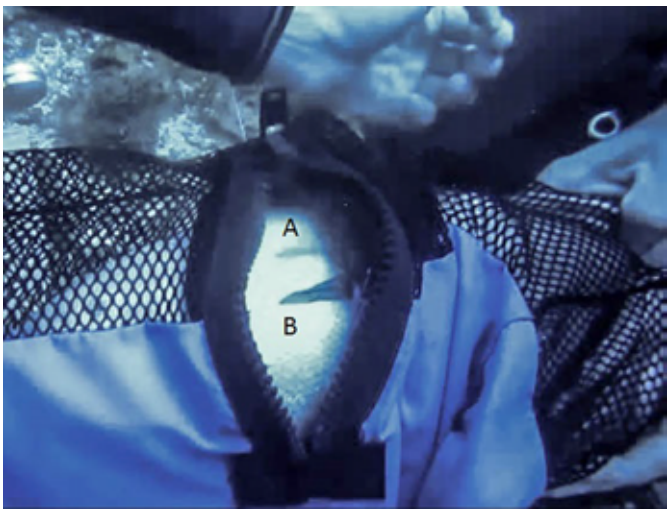


FIGURE 4

Photograph of catch bag with zipper installed giving diver access to fish for surgical procedure: (A) incision site and (B) pelvic fin.



the incision was not made excessively deep in order to prevent exposing or puncturing any of the internal organs or mesenteries. A V16p-4H (69 Hz; 120 s nominal delay) coded-acoustic transmitter (VEMCO, Ltd.) was inserted into the peritoneal cavity, and the incision closed with two to three stainless steel surgical staples (Reflex one skin stapler with 5.7 mm staples). Total length was measured, a fin clip was taken for genetic analysis, and the fish was released. Upon initial release, all fish were observed for a short period to confirm no immediate sign of physiological distress or predation. Once surgical operations were complete, all remaining trap by-catch was released, and the trap was re-baited and closed for subsequent tagging operations.

Fish Tracking

Long-term movement patterns of tagged fish were recorded using a series of 11 omnidirectional VR2 and VR2W (VEMCO, Ltd.) acoustic receivers distributed around BDS at depths between 30 and 75 m. Divers deployed receivers in the months prior to the start of tagging operations. They were mounted on the seafloor using 40 × 40 cm cement pavers and 3/8-inch rebar or attached to weighted buoys and suspended above the substrate. Each receiver continually recorded the presence/absence of tagged fish within an estimated maximum detection radius of 300 m. Divers periodically checked receivers for proper function, and data were downloaded every 6 months. Estimated battery life for each receiver was 12–15 months, with a storage capacity of 300,000 (VR2) to 1 million (VR2W) acoustic detections.

Results

Ten Nassau grouper were tagged in 4 days of diving with all fish tagged in six dives. In most cases, two fish were tagged on each dive, with a maximum of two fish tagged in a single dive and four fish in 1 day. Tagged fish ranged in size from 51 to 80 cm TL, with a mean of 60.1 ± 8.2 cm TL, and the total time required per fish ranged from 10 to 15 min with a mean of 12 ± 2.2 min (Table 1). The majority of the time for each procedure was devoted to fish extraction from the traps. Smaller fish required more time for extraction, as there was more available room inside the trap for these individuals to move and evade extraction techniques. Larger individuals were docile—more so as the expected date of spawning approached and individuals became distended—and required less time for extraction.

Fish behavior immediately following release varied among individuals. Some individuals immediately swam off to seek nearby shelter, where they were no longer visible to the divers. Others casually retreated to join groups of nearby conspecifics, which allowed for slightly longer observation period. In a few instances, tagged fish remained in close proximity to the divers, slowly moving away from the divers a short period after release. In all cases, tagged fish swam away with no visible signs of physiological impairment or discomfort, and their behavior resembled that of nontagged individuals at the aggregation site (Figure 5).

Data confirmed a 100% post-surgery survival rate, with detections recorded for all 10 tags at the main aggregation site for a period of 5 days immediately following surgical tag implantation (Table 1). With one exception, tags to date have been detected for a range of 288–347 days (Table 1), with the latter corresponding to the maximum number of days post-initial tag deployment since data retrieval. One tag (#6) was no longer detected after 44 days. We received a report of a fisherman catching a Nassau grouper with an acoustic tag, which

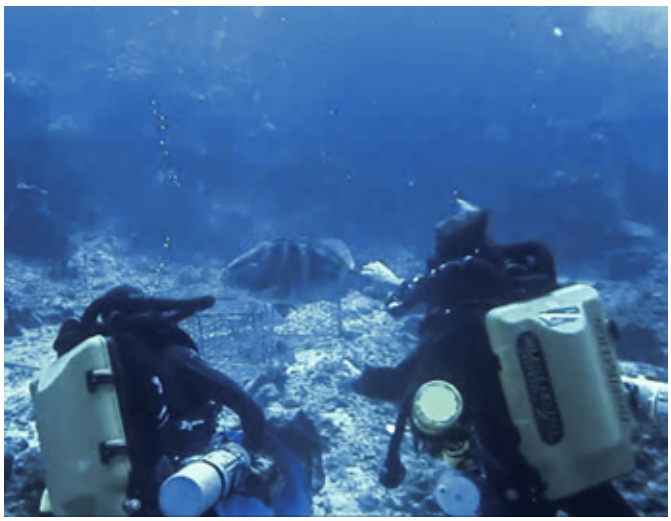
TABLE 1

Total length (cm), total time (min), date tagged, and number of days detected post-surgery for each tag deployed. Total time denotes the time divers commenced fish extraction to the time the fish was released following surgical procedures, recorded to the nearest minute.

Tag No.	Total Length (cm)	Total Time (min)	Date Tagged	Days Detected Post-deployment
1	51	15	March 2, 2013	334
2	61	15	March 2, 2013	347
3	52	14	March 4, 2103	331
4	60	11	March 4, 2013	333
5	60	10	March 4, 2013	288
6	61	10	March 4, 2013	44
7	80	10	March 6, 2013	330
8	63	11	March 6, 2013	330
9	53	14	April 7, 2013	310
10	60	10	April 7, 2013	311
Mean ± SD	60.1 ± 8.2	12 ± 2.2		295.8 ± 90.0

FIGURE 5

Photograph of Nassau grouper release immediately post surgery.



corresponds to this timeline. Unfortunately, we were unable to recover the tag nor confirm this report, presumably from fear of prosecution, as Nassau grouper are a federally protected species in Puerto Rico.

Conclusions

In situ acoustic transmitter implantation has numerous advantages to traditional surface tagging operations. The entire procedure was conducted without removing the fish from the environment within which it naturally occurs, eliminating the need for potentially stressful, long extraction processes and surgical procedures. *In situ* methods promoted highly selective tagging procedures, eliminating bycatch mortality and any undue stress to target species that were not suited for tag deployment. In addition, this methodology allowed for the tagging of fully distended individuals within close proximity to the expected day of spawning, a practice that would likely cause excessive physiological impairment or gamete loss if conducted under traditional surface tagging operations. Diver observation upon release revealed that in all instances tagged fish swam away immediately with no signs of distress, physiological impairment, or predation. *In situ* observation provides a degree of confidence not permitted in surface tagging operations.

The process of conducting acoustic tag deployment entirely *in situ* is not a novel concept (Starr et al, 2000; Lindholm et al., 2005, Feeley et al., 2012). However, this experiment marks the first known practical application of the methodology using CCR technology, where tag insertion was conducted at the same depth and location of capture and at depths within the mesophotic zone. Most importantly, subsequent monitoring revealed a 100% survival rate of tagged individuals—a statistic previously unreported from other studies utilizing similar methodologies (Starr et al., 2000; Lindholm et al., 2005; Feeley et al., 2012). The use of CCR technology significantly increased tagging efficiency by allowing for extended bottom times per dive, reducing the volume of gas needed by each diver, reducing decompression obligations, and reducing the operational cost of diving. Overall, *in situ* methodologies

using CCR and coupled with the extraordinarily high survival rates reported for this study allows the researcher to conclude, with the highest level of certainty, that the tagged individuals behave in a manner that closely resembles that of untagged conspecifics.

Acknowledgments

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