

Methods and Lessons Learnt in the Application of Ultrasonic Telemetry to Coral Reef Fish Movement Studies

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ABSTRACT

There is increasing interest in the use of acoustic telemetry to track the movement of medium-sized marine fishes, particularly coral reef species, as a result of recent improvements in technology which have allowed reductions in the size of both receivers and transmitters. However, acoustic tagging and tracking techniques are still at a developmental stage. In this study we use six medium-size (12 - 46 cm FL) reef fish species (*Clepticus parrae*, *Cephalopholis cruentata*, *C. fulva*, *Lutjanus mahogani*, *Ocyurus chrysurus* and *Kyphosus sectatrix*) to investigate appropriate non-injurious capture and *in situ* retention methods, examine suitable transmitter tag placements (internal versus external) using dummy tags, and test the feasibility of simultaneously tracking up to 20 individuals in a reef environment. The most appropriate capture methods were species-specific and included use of an Antillean fish trap, barbless hook and line, and small mesh net. Clove oil proved to be an effective and fast-acting anaesthetic. Surgical implantation of transmitters into the body cavity proved more successful than external attachment to the dorsal musculature. Super Glue® was found to be effective in closing the incision wound, with full healing in as little as five days. The use of coded transmitters operating on the same frequency allowed for tracking of many individuals simultaneously. However, when several transmitters were in close proximity, the ability of the receiver to decipher individual transmitter codes was greatly reduced.

KEY WORDS: Acoustic tagging methodology, reef fish movement

INTRODUCTION

Patterns of movement and space utilisation by fishes are important to the understanding of population distribution and community structure (Zeller 1997). Such information is also critical in predicting and understanding the effectiveness of marine reserves (MRs) in conserving adult fish biomass, and in providing adult fish biomass to adjacent fisheries (Russ and Alaca 1996).

Information on the pattern of movement and home range size of adult fish is also important for the successful design of MRs. For example, placement of the reserve relative to the position of different habitats; reserve size; and area to boundary ratio will all affect the degree to which adult fish are conserved or 'leaked' to adjacent fishing areas (Holland et al. 1996, Kramer and Chapman 1999). Furthermore, movement patterns are likely to vary among different species.

The need for information on adult fish movement is particularly urgent in coral reef areas where the use of MRs is now being favoured over the more traditional management tools for the effective long-term management of sustainable coral reef fisheries (e.g. Bohnsack 1993, Alaca and Russ 1990, Rowley 1994, Russ and Alaca 1996). However, the majority of information on fish movement and habitat utilisation, by coral reef fish species of commercial importance to fisheries, is inferred from conventional mark and recapture studies which involve the use of fishing gear to recapture marked individuals, or rely on visual observations of marked individuals by SCUBA (visual recaptures) (e.g. Corless *et al.* 1997, see Appeldoorn 1997 for review). The numerous constraints of conventional mark and recapture studies are well documented (e.g. Parker 1990, Watson *et al.* 1993, Appeldoorn 1997). Observer presence and experience may greatly effect the data by means of failure to notice, recognise and record the correct code and position of both the tag and/or the individual fish. Additionally, the recapture of a tagged individual in a particular area at a particular point in time does not preclude its presence elsewhere at any time between release and recapture (Winter & Ross 1982). Consequently this method will always provide an underestimation of actual movement (Appeldoorn 1997). Perhaps because of this constraint, adult reef fish are generally considered to be site-attached with low potential for supplementing fished areas adjacent to MR boundaries (Bardach 1958, Corless *et al.* 1997, Holland *et al.* 1996). Clearly, more complete data on fish movements are needed before any firm conclusions can be drawn on the extent to which MRs will supply adult fish biomass to adjacent fisheries.

Ultrasonic telemetry, which allows continuous tracking of marked individuals, is an ideal tool with which to address the movement and activity patterns of fishes (Winter and Ross 1982, Zeller *in press*). Until relatively recently, this technique was used exclusively on large pelagic species because of the large size and high cost of the early acoustic transmitter tags (Nelson 1990; cited in Zeller *in press*). However, improved technology allowing the production of much smaller and relatively less expensive transmitters now means that this technology can be used on much smaller species, including a wide range of reef fishes. Recent studies have successfully applied ultrasonic telemetry to a number of medium-sized coral reef fishes including *Mulloides flavolineatus* (Holland *et al.* 1993), *Haemulon plumieri* (Tulevech and Recksiek 1994),

Plectropomus leopardus (Zeller 1997, 1998, in press, Zeller and Russ 1998) and *Caranx melampygus* (Holland et al. 1996).

This paper outlines the methods and the lessons learned in the application of ultrasonic telemetry to the study of movement of several other medium-sized coral reef fish species.

METHODS AND RESULTS

Capture Methods

Successful tagging studies require efficient, non-injurious methods of capture. Several methods were investigated in this study to capture medium-sized (12 - 46 cm FL) reef fishes (i.e. creole wrasse, *Clepticus parrae*; grubby, *Cephalopholis cruentata*; coney, *C. fulva*; mahogany snapper, *Lutjanus mahogani*; yellowtail snapper, *Ocyurus chrysurus*; and Bermuda chub, *Kyphosus sectatrix*) with the least damage and stress to both the specimen and the environment. The capture methods and results are described below.

Traps — Traditional un-baited Antillean Z-shaped traps (2 m long x 1 m wide x 0.6 m high, with 1.5" wire mesh) set on sand patches within the reef (5 - 15 m deep) were used in an attempt to capture the target species.

This passive mode of capture was unsuccessful for *C. parrae*, which tend to school above the reef, but was successful for the more benthic reef dwellers *C. cruentata*, *C. fulva*, *L. mahogani*, and *K. sectatrix* and was also occasionally successful for *O. chrysurus* in areas where densities were high. However, repeated soaks in the same area (i.e. > 4 times in 7 days) resulted in *O. chrysurus* and *K. sectatrix* displaying an avoidance behaviour towards the traps.

Once avoidance behaviour was observed, a more active mode of trap fishing was employed for these species. This involved baiting the trap underwater with stale bread placed close to the entrance, and having a diver lying as still as possible, approximately 2 m from the trap. Bread was then crushed and allowed to float in the water near the trap to attract the fish. Once attracted, the diver then slowly moved closer to the trap, eventually rushing the final 0.5 m with arms wide open. This presented the opening of the trap as an avenue of escape, into which the fish fled. This method was very successful for *K. sectatrix* but did not work for *O. chrysurus*.

Fish were removed promptly from traps, since prolonged retention caused additional stress and often resulted in injury.

Nets — A miniature cast net (2 m diam. 1 cm mesh size) was spread in an area in which the targeted fish were frequently seen. The net was either held by two divers or was left hanging at the mouth of a trench in the reef or between two large coral heads. Two to three divers using SCUBA then attempted to shepherd

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the fish in the direction of the net. Once a fish swam into the net, the net was folded over by a diver to prevent escape. This method was successfully used to capture *C. parrae* and *L. mahogani* in highly rugose areas of the reef. The rate of success of this method decreased markedly as rugosity of the reef declined. Attempts to capture *K. sectatrix*, *O. chrysurus* and *C. cruentata* using this method were unsuccessful.

Hook and line — Small barbless No. 9 hooks (2/0 O'Shaughnessy) were baited with strips of fresh ballyhoo (*hemiramphus* sp.), flyingfish (*Hirundichthys* sp.) or clupeids, and dangled near the target species by divers equipped with SCUBA. This was the most successful method of capture for *C. cruentata*. Little success was achieved when this method was employed in the capture of the other target species, and the bait was often rapidly removed from the hooks by schools of *Abudefduf saxatilis* (sergeant major) and *Melichthys niger* (black durgon).

Suction gun — A suction gun was constructed from the cylindrical barrel of an old Van Dorn sampling device (10 cm diam, 75 cm length) fitted with a manual plunger constructed from a circular Perspex disc (9.3 cm diam.) and a 1/2" (1.25 cm) PVC pipe handle that fitted snugly to the interior of the tube, but was free to move up and down. The mechanism of operation is such that when the plunger is depressed and then pulled underwater, a strong suction force is produced which draws the fish into the barrel, facilitating capture. This device, although successful in capturing small-sized benthic reef fishes, was not successful in the capture of any of the target species in this study because of the difficulty in getting close enough to the medium-sized fish for the suction force to be effective.

Retention of Fish

Ex situ retention — Following initial trials of different capture techniques, fish were transported from the reef in sampling coolers containing 40 L seawater and placed in concrete flow-through seawater tanks (0.43 m³) for investigations of anaesthetic and transmitter tag attachment.

Fish were held without feeding for 24 - 48 hr prior to tagging. During this period they were observed for signs of injury from capture and handling, and a crude index of stress was measured by dividing the concrete tank into 4 imaginary sectors and recording the degree of movement between sectors during periods of 20 minutes for each fish, before and after tagging.

None of the three species investigated (*C. cruentata*, *C. parrae* and *L. mahogani*) showed significant differences in their behaviour before and after tagging. However, the death of two *L. mahogani* and two *C. parrae* before

tagging was attributed to stress-related trauma which could not be visually detected.

In situ retention — Once initial trials had been completed, fish were no longer removed from the field, but were retained in underwater shade cages (Figure 1) placed in the reef, for a period of observation and starvation (of at least 24 hrs) prior to tagging. Only one fish was retained in each shade cage at any one time.

Two different sized shade cages (small or large) were used depending on the size of the fish to be held. These followed the basic design of P. Sikkel¹ and were constructed of green or black shade cloth (the type used to provide 80% shade in plant nurseries) covering a nylon rope and 1/2" (1.25 cm) PVC pipe frame measuring 0.5 m x 0.35 m x 0.35 m for small cages and 1 m x 0.70 m x 0.70 m for large cages. A door comprising black Perspex® was placed in the centre (measuring 0.38 m x 0.18 m for small cages and 0.76 x 0.36 m for large cages) of the top face of the cage and this was hinged with plastic cable ties and fastened with nylon rope.

The shade cage served as a means of isolating the fish from its environment (ensuring a period of starvation) as well as providing a safe and secure place to rest (thereby allowing recovery from the stress of capture). The soft material also served to reduce abrasions or injury to the fish when moving suddenly. The shade cages were weighted down with four 1 kg lead weights as well as tied to the traps to prevent relocation by strong currents, and kept in the reef during use. The shade cages worked well for all species tried (i.e. *K. sectatrix*, *O. chrysurus* and *C. cruentata*). However removal of *O. chrysurus* from the cages without losing them proved to be very difficult, even with the use of a safety net placed around the door, owing to their powerful acceleration and strong swimming ability.

Anaesthetic Procedure

Ex situ trials — Two anaesthetics, Quinaldine and clove oil (active ingredient 4-allyl-2-methoxy-phenol; Anderson *et al.* 1997) were used in *ex situ* trials with *C. cruentata*, *C. parrae* and *L. mahogani* in the laboratory holding tanks. Quinaldine was used at a concentration of 0.1 ml/L (2 ml Quinaldine to 20 L of seawater). Clove oil was first dissolved in 70% ethanol (2.4 ml clove oil to 24 ml ethanol) to give a 10% stock solution. This was then added to 20 L of seawater.

Both clove oil and Quinaldine performed equally well at these concentrations in inducing a rapid (in < 2 min) deep anaesthesia in fish of size range 12 – 46 cm FL, from which they recovered in less than 3 minutes.

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However, clove oil was chosen for use in the field because of its non-toxic nature to the environment.

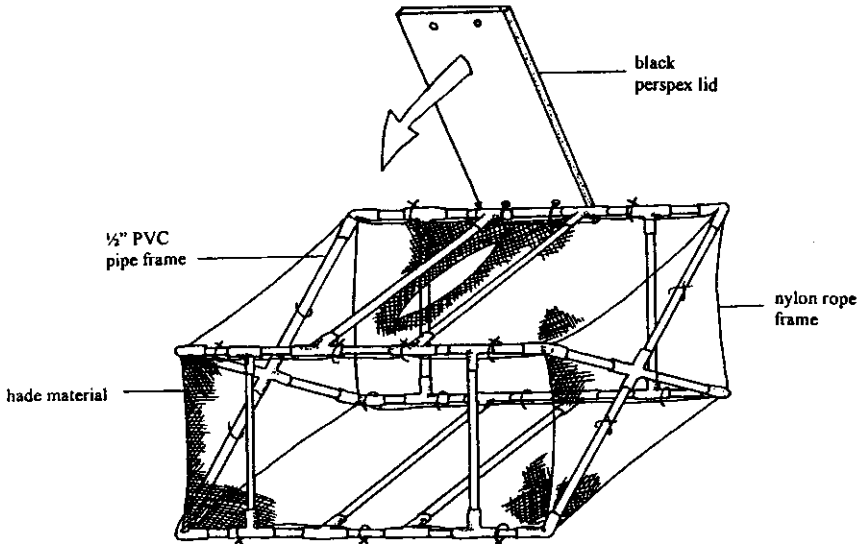


Figure 1. Diagram showing design of the shade cage used for *in situ* retention of reef fish (after P. Sikkel)

Field procedure — Two species, *K. sectatrix* and *C. cruentata* were routinely anaesthetised in the field using the following procedure. The shade cage was first slowly brought to the surface (from depths not exceeding 15 m) by a SCUBA equipped diver, and placed on board the tagging boat (a 5 m inflatable dingy). The fish was then removed from the shade cage and immediately exposed to the anaesthetic by placing it in a sampling cooler containing 20 L of seawater and 24 ml of 10% Clove oil stock solution.

The three induction stages described by Hikasa et al (1986) in Anderson et al (1997) (stage 1: onset of rapid opercula movement; stage 2: erratic swimming and partial loss of equilibrium; stage 3: total loss of equilibrium) were clearly observed in both species and the onset of each stage was recorded by stopwatch. The time to full induction (stage 3) varied among individuals but was negatively correlated with weight in *K. sectatrix*, (Log_{10} induction time (sec) = 4.035 -

0.802 Log₁₀ weight (g); $r^2 = 0.542$, $P = 0.006$) and not significantly correlated to length or weight in *C. cruentata* ($P > 0.05$ in both cases) (Figure 2).

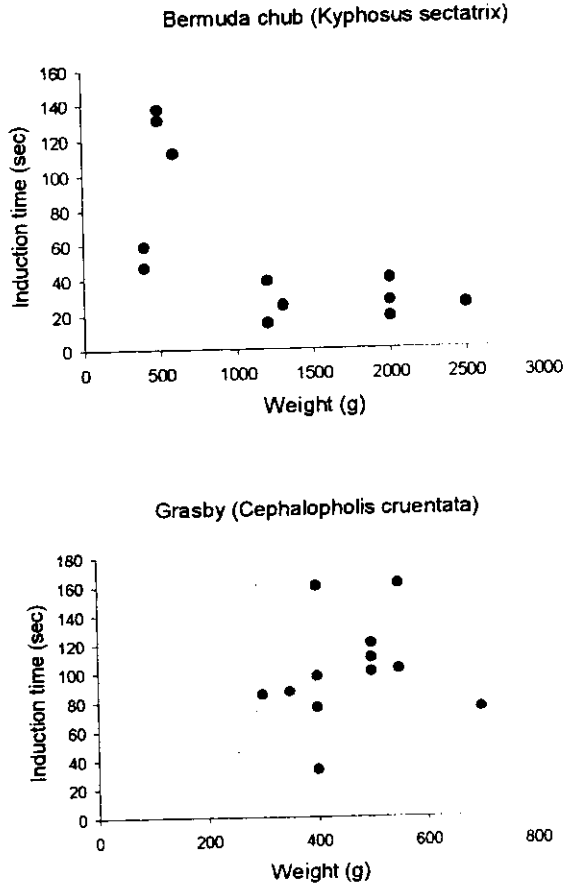


Figure 2. Relationship between fish weight and anaesthetic induction time for the Bermuda chub (*Kyphosus sectatrix*) and grasby (*Cephalopholis cruentata*).

Tag Attachment and Recovery

Dummy tag trials — Both external attachment, through the dorsal musculature, and internal surgical implantation methods of tag attachment were investigated using dummy transmitter tags in initial laboratory-based trials. Dummy tags were made from PVC plastic tubing in two sizes (8 and 11 mm diam., and 18 and 35 mm long respectively) weighted with linseed oil putty and capped at both ends with epoxy resin. The size and weight of the dummy tags approximately

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mimiced those of the Lotek CAFT8-1 (8 mm) and CAFT11-2 (11 mm) transmitter tags.

After induction to the anaesthetic, external attachment was tried using *L. mohagani*, *C. cruentata*, and *C. parrae*. This involved strapping the tag, with 1 mm (diam.) nylon thread, flush to the body of the fish parallel to the dorsal fins as described by Holland et. al. (1996). Total time for both external and internal tag placements never exceeded seven minutes, inclusive of induction time to anaesthetic.

Based on both laboratory and field observations of *L. mohagani*, swimming ability was not diminished by the externally attached dummy transmitter tag. However, repeated attempts were made to remove the tag by chaffing against rocks, and we recorded a 50 % tag loss rate in as little as four days after release into the field. *C. cruentata* showed obvious signs of distress from external attachment of the dummy transmitter tag, manifested by rapid swimming movements and repeated attempts to remove the tag by chaffing it along the side of the holding tank. *C. parrae* showed good recovery from the handling and tagging procedure, but displayed great difficulty swimming. Although they quickly rejoined a school on release into the field, they were unable to keep up with the rest of the school and were soon left behind.

Surgical implantation of dummy transmitters in the body cavity was tried using *L. mahogani*, *C. fulva* and *C. cruentata* (*C. parrae* were considered too small for internal implantation). This procedure involved making an incision immediately posterior to the right pelvic fin, through which the tag was inserted into the body cavity followed by closure of the wound using Super Glue®.

Internally tagged *L. mahogani*, and *C. cruentata* showed good recovery after surgery, with full healing of incision wounds observed in as little as five days. Swimming ability and feeding both appeared unaffected by the operation. The single *C. fulva* used in these trials survived only for a few hours after the surgery owing to the rupture of an extended stomach during implantation. Internal implantation of transmitters was therefore selected for the field study of fish movement.

Field procedure — Acoustic transmitter tags were surgically implanted into 11 individuals of *C. cruentata* and 11 individuals of *K. sectatrix* in the field using the following procedure.

Firstly, the anaesthetised fish was placed on a clean, flat, shaded surface in preparation for the surgery. Then one end of a 3 m long transparent plastic hose (1 cm diam.) was inserted into the mouth and held in place. The other end of the hose was connected to the drain of an elevated cooler containing fresh seawater, thereby allowing a continuous flow of seawater over the gills during the surgical procedure. The fish was also periodically bathed in seawater to prevent

dehydration. In the event that the fish showed signs of recovery before the end of the operation, it was re-exposed to the anaesthetic until complete loss of equilibrium was again observed. The cooler was kept full and details of the operation were recorded.

An incision, slightly larger than the diameter of the transmitter tag, was made equidistant from the ventral fins and the anus and slightly to one side of the central line. This was done with the aid of a size 22 scalpel and a blunt ended seeker. The transmitter was then coated with BNT antibiotic cream and inserted into the body cavity through the incision. The wound was closed with a small quantity of Super Glue®. The fish was subsequently externally marked with a Floy® anchor tag (inserted into the dorsal musculature and anchored between the dorsal spines, midway along the dorsal fin) for easy recognition by divers.

After surgery, the tagged fish was placed in a cooler containing fresh seawater to recover from the anaesthetic and finally transferred to the shade cage, which was subsequently replaced on the reef, using SCUBA. This procedure required a team of three people for smooth running of the operation. Surgical latex gloves were always worn when handling the specimens.

The tagged fish was kept in the shade cage for a further 24 hr during which time the health of the fish could be monitored and the functioning of the transmitter verified. After this the fish was released and tracked.

The species tagged using this method (*K. sectatrix* and *C. cruentata*) showed no apparent discomfort, exhibited fast recovery and retained the transmitters for the duration of the experiment (maximum observed 70 days).

Electronic Equipment

The electronic equipment used in this field study was manufactured by Lotek Marine Technologies Inc., Newmarket, Ontario, Canada.

Transmitter tags — The acoustic transmitter tags were cylindrical-shaped Lotek CAFT 11-2 tags weighing 7.5 g and measuring 11 x 42 mm. Each tag was factory programmed, with a distinct electronic numeric identification code and set to emit an electronic micro pulse every four seconds, once the magnet switch is activated. Lotek estimated transmitter longevity (battery-life) at 90 days.

Receivers — The pulses (acoustic signals) from the transmitter tags were picked up by a directional HPA-O hydrophone fitted with a DAB 45 noise baffle and converted into 76.8 kHz radio frequency signals via a 150 MHz ultrasonic upconverter (UUC). A SRX 400 manually operated telemetry receiver then deciphered these radio signals and displayed signal strength data.

The noise baffle consisted of a windowed sleeve attached over the end of the hydrophone and tightened by two screws. The exposed portion of the

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hydrophone was sensitive to acoustic signals while the covered portion was dampened by the sleeve. The noise baffle served to focus the sensitive portion of the hydrophone in a cone-shaped pattern, providing directionality to the otherwise omnidirectional hydrophone, thereby increasing the ability of the observer to determine the position of the transmitter tag.

There was little problem in following instructions for the assembly of the equipment, but the operations manual for the equipment could only be deciphered by persons knowledgeable in acoustic technology. However, the customer relations department of Lotek Engineering proved excellent, with a trained technician on call to give a step by step tutorial in the operation of the equipment.

Release and Tracking

Following surgical implantation of the transmitter tag and the recovery period, the fish was released, and tracking commenced immediately. Tracking was conducted from the same 5 m inflatable dinghy that the implantation surgery was performed in. The electronic equipment was assembled in the dinghy prior to commencement of tracking (as shown in Figure 3), and dissembled and taken ashore at the end of each tracking session.

The hydrophone was operated by securely fastening it to an oar, which was in-turn then secured to the starboard pontoon of the dinghy. The attachment was such that it allowed the hydrophone to be manually rotated 360 degrees when held vertically or 90 degrees when held horizontally. Thus giving maximum directional coverage for detection of acoustic tag signals both in mid-water (vertical position) and within the reef structure (horizontal position).

Once the receiver equipment was set up and the hydrophone was in place, the SRX receiver was set at five gain steps for initial detection of transmitter signals. When a signal was detected, the hydrophone was rotated to find the direction of maximum signal strength (determined either by loudspeaker output or by the signal strength meter on the SRX receiver). The boat was then maneuvered in the direction of the signal whilst the gain was gradually reduced towards zero. A reduction of the gain effectively reduced the maximum detectable range of the tag. Increased signal strength allowed for distance to an individual fish to be determined so that its position could be recorded within a few metres accuracy.

During tracking sessions, the geographical location of the fish was recorded every 15 minutes using visual hand-held compass fixes on at least three known landmarks. In the event that a fish spent a long time at one position a visual check on the habitat was made using snorkel gear or SCUBA. For the more sedentary *C. cruentata*, the fish position was recorded directly onto a field map of the study area. The field map was divided into 20 x 20 m grid squares and

showed landmarks, reef depth contours and subsurface features which allowed easy recognition of the different grid squares. For night tracking, landmarks that remained lit throughout the night were used as reference points for compass and sextant readings. Light sticks attached to marked reef features were also used to help in the estimation of fish positions. The initial use of a hand-held Magellan GPS was discontinued since the error incurred by selective satellite availability proved to be approximately 20 m (a distance that could place a transmitter in a separate grid square).

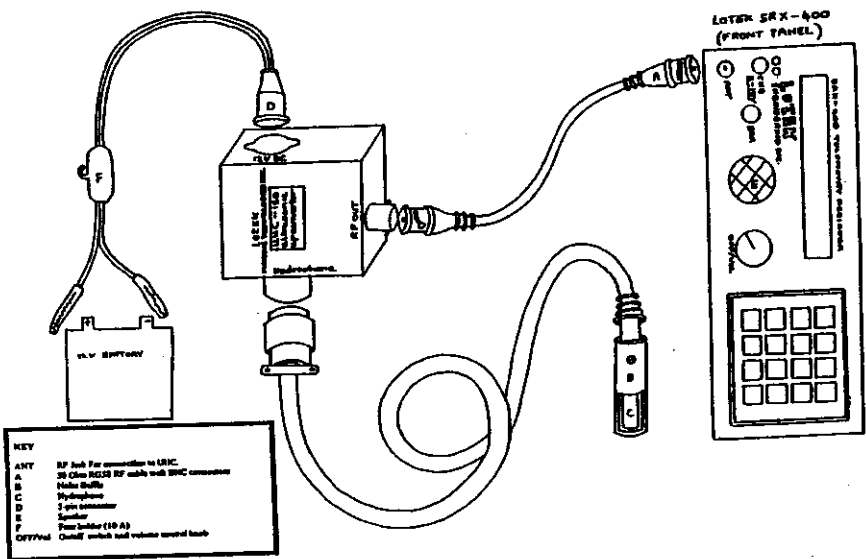


Figure 3. Diagrammatic representation of the assembly arrangement for telemetric receiver equipment used in tracking reef fishes.

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Fish were tracked continuously during the first 24 hours after their release. This labour intensive technique involved using two separate teams of researchers, each undertaking six hour shifts.

Subsequently, each of up to 22 fish were tracked for a minimum of four hours every 24 hours. Those four hours were split between two tracking sessions occurring randomly throughout any 24 hour period. Several different fish were often tracked simultaneously on the same frequency, since each transmitter had a unique identification number.

The tracking procedure generally worked well and signals were detected from tags at depths of up to 30 m and over distances of up to 250 m. This was however considerably less than the 500 m horizontal distance given in equipment specifications. Signal strength was found to diminish drastically when fish entered crevasses in the reef.

Simultaneous tracking of several fish on the same transmitter frequency allowed for the investigation of schooling behaviour. However, signal overlap during schooling was high. The acoustic receiver was often unable to identify individual signals and as a result a large number of error readings were displayed. Another constraint was that signal strength appeared to degrade with battery age. In cases where a transmitter tag was activated three to four weeks before another, operation on the same frequency resulted in the signal of the newer tag masking the signal of the older tag even when separated by distances of up to 100 m.

CONCLUSIONS

Effective, non-injurious methods of capture were species-specific, with baited hook and line being most effective for *C. cruentata*, while Z-shaped Antillean traps were more effective for *K. sectatrix*, *L. mahogani* and *C. fulva*, and a hanging cast net was most effective for the more pelagic *C. parrae*.

Clove oil proved to be an effective anaesthetic with the option of immediate release into the marine environment. Full recovery from the anaesthetic occurred in less than three minutes as reported by Anderson et al. (1997).

External attachment of transmitter tags has been used successfully in several studies (e.g. Tulevech and Recksiek 1994, Begout and Legardere 1995, Holland et al. 1996, Josse et al. 1998). However, it was rejected in favour of surgical implantation in this study after laboratory trials of both methods. Surgical implantation into the body cavity has the advantages of greatly reduced tag loss compared with external attachment, allows a better distribution of the weight near the fish's centre of gravity, and does not create drag forces when the fish is swimming (Mellas et al. 1985). Surgical implantation of transmitter tags into the body cavity proved to be effective in this study for long-term tag attachment in *K. sectatrix* and *C. cruentata*. Only one incident of tag induced mortality was observed out of the 22 fish in which transmitter tags were implanted, although a

number of individuals were lost in the latter stages of the tracking study (i.e. after more than 21 days at large). The probability of stomach and/or swim bladder rupture during tag implantation was significantly decreased by ensuring a period of starvation, and a slow ascent to the surface prior to tagging.

Successes with surgical tag implantation have been reported for several fresh water and diadromous species (e.g. Mellas et al. 1985, Moore et al. 1990); and for coral reef-associated species (e.g. Colton et al. 1983, Zeller in press). However, in the reef-associated species, immediate release of fish after surgery resulted in mortality due to predation, and therefore a 2 - 3 week post-surgery recovery period was suggested to reduce this risk (Zeller in press). The predation-mortality may have occurred because of the relatively substantial incision wounds (2 - 3 cm long) closed by sutures or staples. In this study we found Super Glue® to be effective in closing the small (1 cm) incision wounds without the aid of sutures, and suggest that fish may be safely released 24 hours after tagging. This surgical procedure avoids the problem reported by Tulevech and Recksiek (1994) of exhausting the battery of short-life transmitter tags during post-surgery recovery. Placement of the transmitter tags in the gut was not considered in this study as a result of reported tag losses of up to 60% with this method (Colton and Alevison 1983, Moser et al. 1996).

The use of a mesh shade cage, as suggested by Moore et al. (1990), proved to be a successful means of short-term fish retention. This eliminates the need for seawater flow-through aquarium facilities while in the field and also allowed *in situ* testing of the transmitter tag before release of the fish.

Tracking large numbers of acoustically tagged fish simultaneously proved a monumental task in terms of manpower. Furthermore, transmitter tag signal interference was common with so many tagged fish in relatively close proximity. Reliability of the electronic receiver equipment was also a major issue. Working from a small open boat presented significant challenges for keeping the sensitive equipment dry. Mounting the SRX receiver and UUC in a protective box was not always successful in protecting the equipment from rain and sea spray. This emphasises the need for back-ups at this stage of developing technologies and the need for development of waterproof units.

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