

# Ultrasonic telemetry: its application to coral reef fisheries research

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The importance to fisheries research of understanding movement patterns of fishes is increasingly being recognized (Hilborn and Walters, 1992). Traditionally, mark-recapture studies with external tags constituted the major method of examining movements in fishes (Shepherd, 1988). However, external tagging techniques are known to have several limitations (reviewed by Kearney, 1989) that often cannot be addressed adequately. In particular, data obtained through conventional tagging studies are usually limited to knowledge of a single point of capture, point of recapture, and the straight line distance and time interval between these two events. Such data can be misleading because the exact distance traveled and the patterns of movement are unknown. However, such patterns are of major importance to current research efforts in tropical reef fisheries, including investigations of spawning aggregation events (Samoilys and Squire, 1994; Zeller, 1998), and to the assessment of movements in relation to marine reserves (e.g. Russ and Alcala, 1996).

Ultrasonic telemetry is an ideal tool to address questions of movement and activity patterns of fishes. It can be used effectively under circumstances that limit the use of more traditional methods, yet its suitability for fisheries research is only slowly being realized (Nelson, 1990). Reviews of telemetry in the aquatic environment

are provided by Harden-Jones and Arnold (1982), Hawkins and Urquhart (1983) and Nelson (1990).

Ultrasonic telemetry has been little used on coral reef fishes (e.g. Holland et al., 1993; Tulevech and Recksiek, 1994); most studies have concentrated on sharks (Nelson, 1990). Recent studies, however, have successfully applied the present techniques to the serranid *Plectropomus leopardus* (Zeller, 1997, 1998; Zeller and Russ, 1998), the primary target species of line fisheries on the Great Barrier Reef, Australia (Kailola et al., 1993).

The aims of this study were the assessment of ultrasonic telemetry in the coral reef environment and the application of the technique to *P. leopardus*. The first objective was the determination of a suitable transmitter placement technique and anesthetic. The second objective consisted of a field evaluation of ultrasonic telemetry. The final objective comprised tracking trials of *P. leopardus*.

## Materials and methods

### Study location and ultrasonic telemetry equipment

The study was conducted during 1993 at Lizard Island Research Station, northern Great Barrier Reef, Australia (lat. 14°40'S; long. 145°28'E). The telemetry equipment consisted of V8-2L transmitters and a VR60 receiver linked to a direc-

tional 50–80 kHz hydrophone (Vemco Ltd, Halifax, Canada).

### Evaluation of fish anesthetics and transmitter placement techniques

Fish (size range: 34.0 cm–52.5 cm fork length [FL]) were caught on hand lines. Aquarium facilities included 500-, 1000-, and 2000-L tanks with continuous flow-through water supply. Stocking densities did not exceed 2 fish/500 liters. Specimens were retained for an acclimation period (1–3 days), and were not fed for 24 hours prior to the experiments.

The first concern was that of procuring a successful, deep anesthesia that allowed a gentle recovery in the fish. Three anesthetics were tested: 1) Hypnodil™ (Janssen Pharmaceutica, active ingredient: metomidate) was used at a concentration of 7 mg/L (Mattson and Rippe, 1989); 2) phenoxyethanol was used at a concentration of 0.5 ml/L (Mattson and Rippe, 1989); and 3) MS-222 (tricaine methanesulfonate) was used at a concentration of 80 mg/L (Thomas and Robertson, 1991).

Three methods were used to attach telemetry units to the animals: 1) stomach placement (force feeding) by means of oral insertion of the transmitter into the stomach (e.g. Holland et al., 1992)—the least intrusive method, neither resulting in external protrusion of the unit, nor requiring surgery; 2) external placement by attaching transmitters directly to the dorsal musculature (Holland et al., 1996); and 3) internal placement by inserting transmitters into the body cavity as described by Hart and Summerfelt (1975) and Holland et al. (1993). Surgical implements were disinfected in ethanol and soaked in Tamodine™, a fish-antiseptic solution (Vetark Professional, Winches-

ter, UK). The incision was placed parallel to the midventral line on the left hand side of the fish, 2 cm anterior to the anus and 1–2 cm lateral of the midventral line. Three to four rows of scales were removed from the area of planned incision, and the area was cleaned with Tamodine™. A 2–3 cm anterior-posterior incision was made into the body cavity. Disinfected transmitters were coated in antiseptic cream prior to careful insertion into the body cavity. The incision was closed with 6–8 surgical staples (Ethicon Proximate III™) as suggested by Mortensen (1990), the area was cleaned with Tamodine™, and the fish given an intraperitoneal injection of antibiotic (tetracycline 50 mg/kg of fish, McFarlane and Beamish, 1990), before being returned to the aquarium tanks.

**Aquarium experiments** Owing to limited aquarium space, it was not possible to evaluate all combinations of anesthetics and placement methods simultaneously. Hence two separate experiments were conducted. Experiment A examined the least intrusive transmitter placement technique (force feeding) with all three anesthetics. In experiment B the two intrusive techniques (external attachment and insertion into the body cavity) were tested in conjunction with the two most suitable anesthetics.

**Experiment A: force feeding** All three anesthetics were tested with force feeding to assess differences in retention times of transmitters due to the anesthetic agent. It was hypothesized that use of the hypnotic agent Hypnodil™ (metomidate) would result in the longest retention times owing to the low stress levels caused by this compound (Thomas and Robertson, 1991). *Plectropomus leopardus* regurgitate stomach contents easily, particularly when exposed to stressful conditions.<sup>1</sup>

Twenty-one specimens of *P. leopardus* (size range: 39.0 cm–52.5 cm FL) were assigned randomly to the three anesthetic treatments ( $n=7$ ). Individual fish were anesthetized, tagged with T-bar anchor tags for identification, their fork length was measured, individually numbered transmitters were inserted into the stomach, and fish returned to the aquaria for recovery. Fish were fed daily. Aquaria were examined for regurgitated transmitters every two hours during daytime and once during the night. The start of each inspection was taken as the maximum retention period for any recovered units.

**Experiment B: external attachment and surgical insertion** The suppression of a stress response due to the use of metomidate (Hypnodil™) makes this a valuable drug for the routine handling of fishes (Thomas and Robertson, 1991). However, slightly elevated levels of the stress hormone corticosteroid are considered beneficial for resistance to trauma, thus making metomidate less suitable in situations involving stress, such as surgical procedures (Thomas and Robertson, 1991). Given the nature of the intrusive manipulations performed in experiment B, the use of Hypnodil™ was discontinued.

Experiment B compared two anesthetic agents (phenoxyethanol and MS-222) and two transmitter placement methods (external attachment and surgical insertion) in a full factorial design. The transmitter placement factor consisted of five treatment levels: external and internal placement treatments, external and internal placement controls, and a routine handling control. All fish received the same preliminary handling: fish were anesthetized, weighed (to the nearest 50 g), measured (fork length), and tagged externally. Routine-handling control fish were returned to the aquaria. The external attachment treatment consisted of attaching transmitters *sensu* Holland et al. (1996), and injecting an antibiotic intraperitoneally (tetracycline 50 mg/kg of fish, McFarlane and Beamish, 1990), before fish were returned to the aquarium. The procedure lasted 5–8 minutes. External-attachment control fish underwent the same procedure, but a transmitter was not attached after puncturing the musculature, and the fish were returned to the aquaria after eight minutes. The internal placement treatment consisted of surgical insertion of the transmitters into the body cavity as described above. The procedure took 15–20 minutes. Internal placement control fish were treated identically, but no transmitter was placed in the body cavity prior to closure of incisions. Fish were returned to the aquaria after 20 minutes.

Twenty *P. leopardus* were used (size range: 34.0–50.7 cm FL), resulting in two random replicates per treatment combination. Fish were fed daily, and each individual was examined visually without handling, to record general condition, behavior, feeding and wound status. The experiment was terminated after 24 days because gas-bubble-trauma (Weitkamp and Katz, 1980) affected the behavior (lethargy and cessation of feeding) of fish from all experimental groups. Upon termination, wounds were examined and classified as aggravated (ripped, or inflamed [secondary infection]), partially healed (40–50% wound closure), or healed (possibly minor inflammation). Subsequently, transmitters were removed and fish were weighed.

<sup>1</sup> Davies, C. 1992. Cooperative Research Centre for Reef Research, James Cook University of North Queensland, Townsville, 4811, Australia. Personal commun.

**Data analysis** Data from experiment A were analyzed for differences in retention times between anesthetic agents by using a single-factor analysis of covariance (ANCOVA). Fork length was used as a covariate to account for any effect of size of fish on the retention times. Assessment of experiment B was based on the observations made during and after the experiment. The change in weight during experiment B was analyzed by using a two-factor analysis of covariance (ANCOVA). Occurrence of gas-bubble-trauma was treated as a covariate. Underlying assumptions were evaluated prior to analyses (Sokal and Rohlf, 1981).

#### Field evaluation of ultrasonic telemetry in the coral reef environment

The extensive use of radio telemetry in wildlife research has produced procedures to assess the accuracy (bias and precision) of telemetry reception (White and Garrott, 1990). However, no such procedures appear to be published for ultrasonic telemetry. Given the paucity of studies with ultrasonic telemetry in the coral reef environment, a standardized field test using stationary transmitters was undertaken. Given that wind and sea-state influence the detectability of sound transmission in water (Jellyman et al., 1996), any effect of direction of prevailing wind on detectability or directional bias of observed sound signals needed to be assessed. Thus, the objectives of this field evaluation were 1) to establish the accuracy of directional bearings in relation to prevailing wind direction; 2) to evaluate observer bias and precision of bearings in relation to wind direction; and 3) to determine the optimal angle for cross-bearings, and the optimal distance between tracking boat and signal in order to obtain position estimates that minimize error polygons.

**Experimental methods** An ultrasonic transmitter (V8-2L, Vemco Ltd) was attached 15 cm above the substratum to a surface buoy in 4–6 m water depth. Three 200-m transects were run from the moored transmitter in relation to the prevailing wind direction:

- 0°: transmitter located directly upwind from any position on the transect.
- 45°: transmitter located at 45° downwind from the prevailing wind direction.
- 90°: transmitter located at right angle to the prevailing wind direction.

Marker buoys were positioned at 25-m intervals along each transect, starting at 50 m from the transmitter location (i.e. from 50 to 200 m), resulting in

21 positions for the three transects. Replicated compass bearings to the transmitter were obtained for each position by using the following protocol: the field of view of the observer was restricted to the tracking receiver for the duration of the experiment; an assistant anchored the boat at the marker buoys in random order and recorded the true compass bearing to the transmitter. The observer determined the perceived maximum directional signal strength by using the directional hydrophone. The corresponding compass bearing of the directional hydrophone (observed bearing) was recorded. The direction of the hydrophone was changed haphazardly by the assistant and the procedure was repeated. Six replicate bearings were taken at each of the seven distances on the three transects. The procedure was repeated by the second observer for a total of 252 observed bearings.

**Data analysis** Data were assessed for accuracy, bias, and precision (White and Garrott, 1990). Bearing error polygons were determined by using the largest and smallest directional bearing observed at each distance-marker buoy for any combination of two transects (0°–45°, 45°–90°, and 0°–90°). Thus, the error polygon parameters obtained (polygon area and maximum diagonal dimension) represented the largest possible error polygon estimates. Only bearing pairs from equidistant locations were used for polygon determination. Statistical analyses included Kruskal-Wallis nonparametric ANOVA and paired *t*-test. All data were examined for violations of underlying assumptions prior to analysis (Sokal and Rohlf, 1981) and data  $\log_{10}$  transformed where applicable. Given that the angular scale of measurement represented only part of a circle, and absolute direction of angles was of no interest, data were treated as linear (Cain, 1989).

#### Field tracking trials

To verify the findings from the aquarium study, the two most suitable long-term transmitter placement techniques (external and internal placement) were tested under field conditions (MS-222 used as anesthetic). Two *P. leopardus* (43.1 cm and 58.9 cm FL) were equipped with external transmitters and released at their capture site after a 15-min recovery period. In the second field trial, internal transmitters were placed in two specimens (59.0 cm and 42.9 cm FL). Fish were released at the capture site after recovery from anesthesia.

The basic tracking technique described by Holland et al. (1985, 1992) was used. Exact positions of fish equipped with transmitters were determined by visual

triangulation (White and Garrot, 1990) by using identifiable landmarks and reef features. Triangulation involves the estimation of the location of a transmitter by using two or more directional bearings obtained from a known location (White and Garrot, 1990). Visual triangulation uses visible land and reef features that can be identified from maps or aerial photographs, in conjunction with directional bearings to identify the location of the transmitter.

## Results

### Evaluation of fish anesthetics and transmitter placement techniques

**Experiment A: force feeding** Gastric retention times of force-fed transmitters ranged from 18 to 216 hours; longer retention periods were documented for larger fish (Fig. 1,  $r=0.621$ ,  $n=21$ ). Mean retention times differed between anesthetic agents used (ANCOVA,  $F_{2,17}=4.762$ ,  $P=0.023$ ). The use of MS-222 resulted in a shorter retention time compared with either phenoxyethanol (SNK,  $P=0.004$ ) or metomidate (SNK,  $P=0.001$ ). Mean retention times did not differ with the use of either phenoxyethanol or metomidate (SNK,  $P=0.216$ ). On average, fish anesthetized with MS-222 retained the transmitters for 42.0 hours ( $\pm 9.7$  SE), in contrast with fish anesthetized with phenoxyethanol (118.0 hours [ $\pm 17.2$  SE]) and metomidate (147.4 hours [ $\pm 23.8$  SE]).

**Experiment B: external attachment and surgical insertion** The change in weight of fish over the duration of the experiment differed significantly between transmitter placement treatments (ANCOVA,  $F_{4,9}=5.309$ ,  $P=0.018$ ). Fish carrying external transmitters displayed a substantial reduction in weight compared with fish in all control groups that gained weight, whereas the internal treatment group showed no change in mean weight during the experiment (Fig. 2). Fish with external transmitters were observed spending a lot of time rubbing the attached transmitter against the substratum. Furthermore, most fish started feeding within two days, except for fish in the external treatment group and

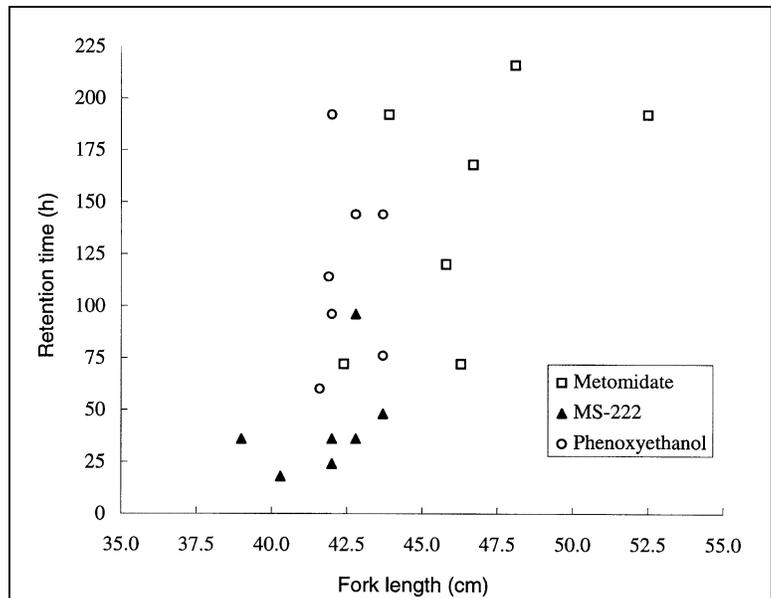


Figure 1

Correlation of gastric retention time of force-fed transmitters versus fork length of *P. leopardus*, as determined in experiment A ( $n=21$ ,  $r=0.62$ ). The three anesthetic agents are indicated.

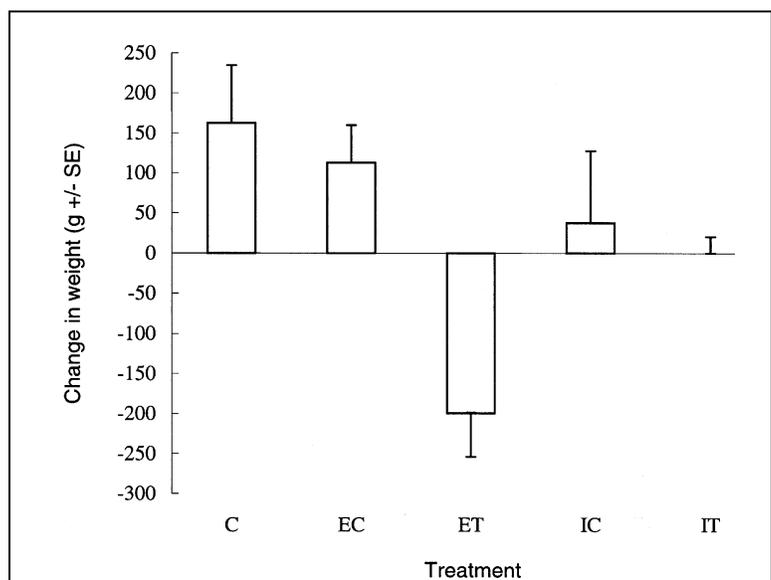


Figure 2

Change in mean weight (before treatment to after treatment) of *P. leopardus* as determined in experiment B. Note: negative weight indicates a loss in weight and vice versa. Presented are means ( $g \pm SE$ ). Treatments: C = routine handling control; EC = external attachment control; ET = external attachment treatment; IC = internal placement control; IT = internal placement treatment.  $n = 20$ .

those anesthetized with phenoxyethanol in the internal control group (Table 1).

Table 1

Observations for aquarium experiment B. Treatment: C = routine handling control; EC = external attachment control; ET = external attachment treatment; IC = internal placement control; IT = internal placement treatment. Anesthetic: MS = MS-222; P = phenoxyethanol. "—" indicates that specimen never resumed feeding after treatment.

Treatment	Type of anesthetic	Level of anesthesia achieved	Day of first feeding	Affected by gas-bubble-trauma	Wound status
C	MS	deep	2	Y	—
C	MS	deep	2	N	—
C	P	twitching	2	N	—
C	P	deep	2	N	—
EC	MS	deep	—	Y	partially healed
EC	MS	deep	2	N	healed
EC	P	deep	—	Y	partially healed
EC	P	twitching	2	Y	healed
ET	MS	deep	10	Y	aggravated
ET	MS	deep	—	Y	aggravated
ET	P	deep	10	N	aggravated
ET	P	twitching	7	N	aggravated
IC	MS	deep	2	N	healed
IC	MS	deep	2	N	healed
IC	P	deep	7	Y	healed
IC	P	deep	7	N	healed
IT	MS	deep	2	N	healed
IT	MS	deep	2	N	healed
IT	P	deep	2	N	healed
IT	P	twitching	—	Y	healed

Observational records illustrated some differences in the effects of the anesthetics. All fish anesthetized with MS-222 attained deep anesthesia, whereas 40% of fish exposed to phenoxyethanol did not (Table 1). This resulted in twitching of the animal during treatment. Occasional cramp-like convulsions were also observed in some of these specimens during the early recovery period.

Examination of placement wounds after the termination of the experiment revealed consistent patterns (Table 1). The incisions made into the body cavities of internal control and internal treatment fish were healed (some minor inflammations did exist). The puncture wounds through the dorsal musculature of fish with externally attached transmitters were aggravated and in some cases enlarged due to repeated attempts to dislodge the transmitters. Wounds on external control fish were either healed or partially healed, and there were no further signs of aggravation.

#### Field evaluation of ultrasonic telemetry in the coral reef environment

Evaluation of bearing errors (true bearing-observed bearing) by transect (0°, 45° vs. 90°) indicated a significant difference in mean bearing error between

transects (Kruskal-Wallis  $H_{2,252}=22.102$ ,  $P=0.000$ ). The 90° transect displayed the least bias (mean= $-1.50^\circ \pm 0.7163^\circ$  SE), the 0° transect had slightly larger, positive bias (mean= $2.09^\circ \pm 1.077^\circ$  SE), and the 45° transect showed strong, negative bias (mean= $-5.43^\circ \pm 1.021^\circ$  SE).

Owing to the differences in bias and accuracy between transects, observer differences in bias were examined separately for each transect. Bias differed between observers for the 0° transect (Kruskal-Wallis  $H_{1,84}=6.238$ ,  $P=0.013$ ); observer two showed positive bias compared with observer one (Fig. 3). Observers did not differ significantly in bias for the 45° transect (Kruskal-Wallis  $H_{1,84}=0.209$ ,  $P=0.648$ , Fig. 3), or the 90° transect, (Kruskal-Wallis  $H_{1,84}=2.603$ ,  $P=0.107$ , Fig. 3).

Paired *t*-tests were used to compare precision estimates (SD) between observers for each transect separately. No significant observer difference in precision was detected: 0° ( $t_6=1.2599$ ,  $P=0.2545$ ), 45° ( $t_6=1.5819$ ,  $P=0.1648$ ) and 90° ( $t_6=0.6859$ ,  $P=0.5184$ ).

Evaluation of the parameters of maximum-error polygons indicated some clear patterns, both with respect to distance between sound source and tracking boat and with respect to angular combination of position bearings taken. The 0°–45° transect combina-

tion generally produced the largest error polygon area and length estimates, regardless of distance between sound source and tracking boat (Fig. 4). The 45°–90° transect combination produced similar results, with the exception of 50 m distance, where this combination resulted in the smallest polygon area and length (Fig. 4). Overall, however, the 0°–90° transect combination consistently resulted in the smallest polygon parameter estimates, irrespective of distance from which bearings were taken (Fig. 4). The best results for this angle combination were obtained at distances of 50 to 75 m from the sound source, with maximum-error polygon diameters of 58.5 m and 34.5 m, respectively.

**Field tracking trials**

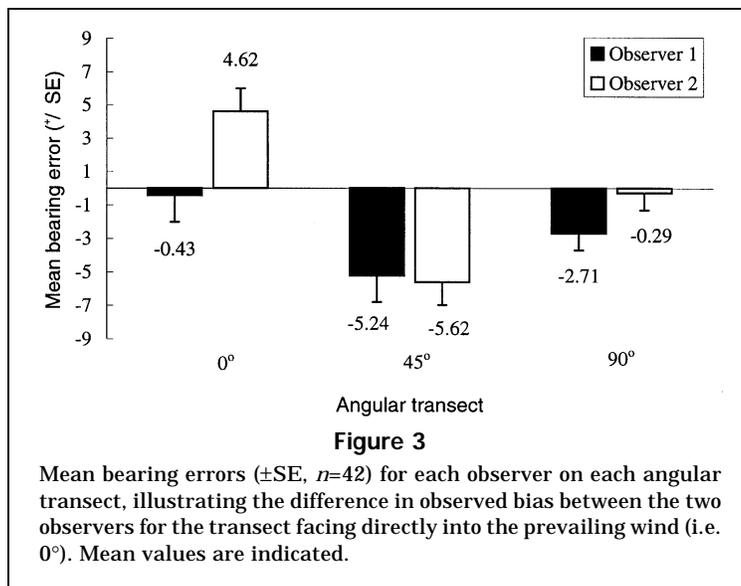
The two fish tagged with external transmitters did not travel far. The larger specimen traveled less than 25 m, whereas the smaller fish moved a maximum of 60 m. Both fish spent considerable time inside the shelter of the reef matrix, with the result that a low acoustic signal was received, accompanied by reduced reception angles. This behavior was confirmed during visual observations. The tracking trial was terminated after four days, and both fish were collected. Both specimens exhibited aggravated external wounds. Repeated dislodgment attempts, or snagging of the external transmitter on reef substratum had resulted in enlarged external wounds and loosened transmitters.

The two fish with surgically implanted transmitters took shelter inside the reef matrix immediately after release (observed on snorkel) and were not resighted. The signal weakened once the specimens had taken shelter, and remained weak for 48 hours. By the third day both signals were received strong and clear in the same location. Visual confirmation indicated that both transmitters were inside a whitetip reef shark (*Triaenodon obesus*). The immediate, postsurgery release of *P. leopardus* had resulted in mortality due to predation within 48–72 hours after release.

**Conclusions and recommendations**

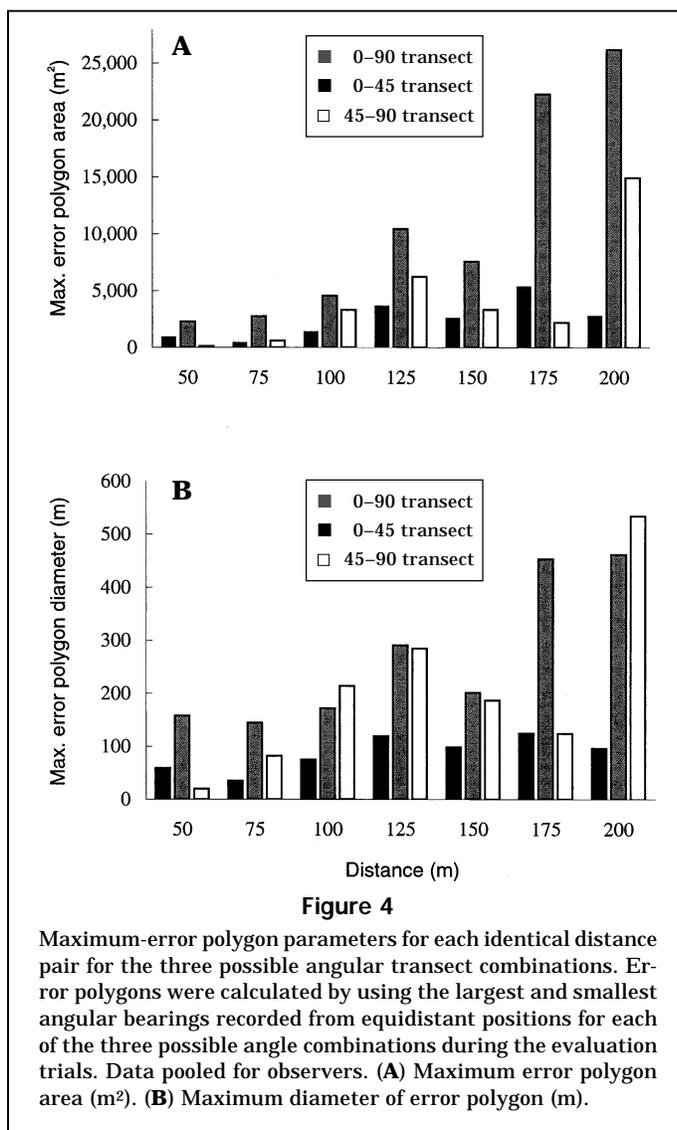
**Transmitter placement and fish anesthetics**

The aquarium experiments illustrated that, although surgical insertion is technically the most difficult method, it provided the least side effects



**Figure 3**

Mean bearing errors (±SE, n=42) for each observer on each angular transect, illustrating the difference in observed bias between the two observers for the transect facing directly into the prevailing wind (i.e. 0°). Mean values are indicated.



**Figure 4**

Maximum-error polygon parameters for each identical distance pair for the three possible angular transect combinations. Error polygons were calculated by using the largest and smallest angular bearings recorded from equidistant positions for each of the three possible angle combinations during the evaluation trials. Data pooled for observers. (A) Maximum error polygon area (m²). (B) Maximum diameter of error polygon (m).

and should be selected for longer term studies, especially for bottom-associated reef fishes. Wound closure was observed within 14–21 days, and subsequent research (Zeller, 1997, 1998) illustrated that secondary infections were reduced with the use of a broad-spectrum bactericide (Myxazin™, Waterlife Research Industries, Longford, UK) and oral antibiotic (tertracycline). After the initial occurrence of gas-bubble-trauma (Weitkamp and Katz, 1980), the aquarium water was filtered through gravel prior to use. No further incidents of gas-bubble-trauma occurred (Zeller, 1997, 1998).

Releasing the experimental tracking specimens immediately after intrusive surgery meant releasing an injured fish that became a prime target for reef fish predators. All subsequent tracking specimens were retained for a recovery period in aquaria until the implant incision was healed (2–3 weeks). This procedure proved to be successful during subsequent studies and no further fatalities due to predation were observed (Zeller, 1997, 1998).

This study demonstrated clearly that external attachment of transmitters is not a viable option for *P. leopardus* because fish are disturbed by the external package and attempt to dislodge the devices. However, a study on a reef-associated carangid, a species with a more pelagic lifestyle, reported successful use of external transmitters (Holland et al., 1996). This finding illustrates the necessity of evaluating transmitter placement techniques in relation to species and species-specific habits.

Force feeding was demonstrated to be of use with *P. leopardus* only for short-term duration not exceeding a few days. Even the longest retention time recorded (216 hours) was considered unsatisfactory for longer term tracking studies (Zeller, 1997, 1998).

Tricaine methanesulfonate (MS-222) was the best anesthetic because it induced a successful loss of sensation and a gentle recovery, with the potential additional benefit of improved recovery from physical trauma because it elevated levels of the corticosteroid hormone (Thomas and Robertson, 1991). Furthermore, unlike with phenoxyethanol, Losey and Hugie (1994) did not observe any negative effects with MS-222 in regard to the olfactory sense. The present findings suggest that MS-222 represents the most suitable anesthetic for the surgical placement of transmitters.

#### Ultrasonic tracking of coral reef fishes

The following considerations will improve position estimation for tracking studies on coral reefs, and have been applied successfully (Zeller, 1997, 1998; Zeller and Russ, 1998): manual tracking with visual triangulation should be conducted by taking bear-

ings at approximately right angles (90°) to each other and with approximate distances of 50–75 m between vessel and transmitter. Bearings taken at angles less than 90°, or taken at sharp angles to the prevailing wind, should be avoided. These considerations will result in minimal directional bias of bearings and ensure maximum accuracy and precision of estimates. If uncertainty about location or directionality of signal exists, it is suggested that “ground-zero tracking” (Nelson, 1990) be used to verify exact location of tracking specimen.

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