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Validating variation in radio-signal strength as an index of aquatic fauna activity

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Abstract. Studying biological rhythms of activity and determining the external factors that influence behaviour of animals can be challenging in many aquatic habitats. We investigated the validity of using variations in radio-signal strength to quantify changes in activity of radio-tagged aquatic fauna on a small spatial scale under controlled conditions in the field. We monitored short-term activity (<1 min) of two aquatic species, rainbow trout (*Oncorhynchus mykiss*) and Murray River crayfish (*Euastacus armatus*), that differ markedly in their primary mode of movement. Simultaneous video monitoring confirmed that active and inactive periods for both species could be accurately determined by radio-telemetry, as were specific behaviours exhibited by trout. We were also able to quantify activity based on different radio-tag (coil and trailing whip antennas) and receiving antenna configurations (yagi and gap-loop antennas); however, we recommend use of control tags to provide reference data. Variation in radio-signal strength represents a valid means of monitoring activity of moderately site-attached aquatic species.

Introduction

Remote telemetry is rapidly improving our understanding of animal movement in aquatic ecosystems (for reviews see Heupel *et al*. 2006; Cooke 2008). Telemetry stations enable continuous monitoring of fish movement where gates (cf. Heupel *et al*. 2006) are commonly used to record large-scale fish movements. In linear systems (i.e. streams), fish have been recorded passing stations, entering or congregating near in-flows (e.g. O'Connor *et al*. 2005) or leaving a study area (e.g. Ebner and Thiem 2009). Alternatively, arrays (multiple receiving stations) have been used for complete coverage of short channel sections in linear systems, generally downstream of discharge outlets (e.g. Cooke *et al*. 2004) and in fish passage facilities (Bunt *et al*. 1999, 2000).

Monitoring fish activity provides insight into specific behaviours and represents an important link in bioenergetics models; however, it can be challenging to quantify under field conditions (Boisclair and Leggett 1989). Where tagged fish reside within the detection range of an antenna for extended periods (e.g. occupying small home ranges), activity can be determined by radio-telemetry. Radio-transmitters equipped with motion-sensitive switches (that increase pulse rate in relation to the movement intensity of the tag) can be used to record activity and inactivity (e.g. Beaumont *et al*. 2002; Jellyman and Sykes 2003; Karppinen and Erkinaro 2009). Karppinen and Erkinaro (2009) contend that this method is advantageous compared with use of electromyogram transmitters, particularly in terms of the invasive surgical techniques associated with the latter. A third method of monitoring activity relies upon the use of variation in the strength of radio-signals from standard radio-transmitters to indicate activity of individuals. Applications of this method are relatively common (Grigg *et al*. 1992; Baras *et al*. 1998; Young 1998; David and Closs 2001; Cooke *et al*. 2002; Hiscock *et al*. 2002; David and Closs 2003; Robertson *et al*. 2003, 2004; Thiem *et al*. 2008). Used in conjunction with continuous data loggers, the method is generally employed to describe diel activity patterns (e.g. David and Closs 2001), although it has also been used to correlate fish activity with discharge (Robertson *et al*. 2004) and parental care (Cooke *et al*. 2002). A major advantage of this method is that it can be based on implantation of small radio-tags (<1 g) that do not require motion-sensitive tilt switches, thus increasing the range of species and size classes to which this technique is applicable. The underlying assumptions of this technique are that constant radio-signal strength reflects a lack of animal motion and varying radio-signal strength reflects activity. While these assumptions have been tested by David and Closs (2001), whereby a dead fish implanted with a transmitter was artificially moved and a relationship inferred, validation on live fish is still required.

Constant signal strength may reflect slow but constant movement by an animal, saturation or insensitivity of gear to small scale changes, or an inappropriate temporal resolution of sampling. In the case of the latter, a single bout of feeding lasting less than 10 s (Beaumont *et al*. 2002) may not be detected by

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pooling data at more coarse intervals, for example 10-min intervals (David and Closs 2001). Furthermore, other factors may affect interpretation of the data. For instance, environmental conditions may also affect radio-signals. David and Closs (2001) found that radio-signal strength was inversely correlated with temperature, though within a sampling unit (10 min) there was negligible variation.

We hypothesised that variation in radio-signal strength at a minimum provides a measure of aquatic animal activity and inactivity. We proposed to test this through applications involving two taxa (a fish and a crayfish) with substantially different modes of locomotion, to make generalisations about the applicability of our results. Additionally, we aimed to determine whether variation in signal strength can be used to quantify the intensity of animal activity rather than producing only binary data.

Methods

Study site

An artificial enclosure was situated on the stream bank, adjacent to the Cotter River (35°22′S, 148°53′E), Australian Capital Territory, Australia. The study site was chosen to simulate local habitat conditions known to affect radio-transmitter signal strength (i.e. incised, bedrock-dominated valleys: Broadhurst and Ebner 2007). The enclosure consisted of a heavy-duty circular plastic tub (2 m diameter, 1 m height, ~2000 L) and was filled with river water (mean ± s.e.: water temperature 5.21 ± 0.15°C, conductivity 55.36 ± 0.10 μS cm^{-1}, pH 7.64 ± 0.02, and turbidity 0.77 ± 0.21 NTU).

Radio-tagging

Rainbow trout (*Oncorhyncus mykiss* Walbaum) and Murray River crayfish (*Euastacus armatus* von Martens) were used to simulate differing movement characteristics (rainbow trout: active, fast-swimming species; Murray River crayfish: slow-moving, relatively sedentary benthic species: Ryan *et al*. 2008). Rainbow trout were sourced from a local trout hatchery (Gaden Trout Hatchery). Murray River crayfish were captured using baited hoop nets from the Murrumbidgee River (35°26′S, 149°04′E), Australian Capital Territory. Both species were held in aquaria before tag attachment and housed individually to prevent aggressive interactions.

Two rainbow trout were fitted with radio-tags in this experiment. These individuals were anaesthetised with 0.5 mL Alfaxan (Jurox, Rutherford, Australia) per 10 L of water. Two radio-tag models were used in this study, each with an individual frequency in the 150–151 MHz range. Both radio-tag models were of sufficient size to contain internal microprocessors, eliminating any potential radio-signal strength fluctuations with changing temperature (see David and Closs 2001). The first model, an internal body implant radio-tag with a coiled antenna (model F1215, weight 9 g in air, Advanced Telemetry Systems (ATS), Isanti, USA), was inserted via a 2-cm ventro-longitudinal incision into the peritoneal cavity of one individual (Tc: 431 mm TL, 981.1 g). Two sutures (size 2/0 non-absorbable suture, Braun, Germany) were used to close the incision and a temporary skin adhesive, Vetbond (3M, St Paul, MN, USA), was used to hold the incision closed. Similar surgical methods (with the exception that the trailing whip antenna exited the body wall using a modified cannular) were used to implant the second radio-tag, an internal body implant with a trailing whip antenna (ATS model F1820, weight 8 g in air), into a second individual (Tw: 394 mm TL, 771 g). Both trout received an antibiotic injection of Baytril administered to the nape. The trout recovered in aquaria following surgery, with a total operation time (from initial immersion in anaesthesia to full recovery) of 24 and 25 min for coil and whip antennas, respectively.

A coil antenna radio-tag (ATS model F1215, weight 9 g in air) was externally mounted to the dorsal carapace of a single Murray River crayfish (Cc: OCL 105.7 mm, weight ~500 g), using a rapid-set epoxy resin (Araldite 5 min epoxy adhesive; Araldite®, Switzerland). The crayfish was removed from its holding tank and placed in a small open container with enough water to keep the underside of the body moist. Once the carapace had dried, the radio-tag was set in place using two coats of epoxy resin. The crayfish was held in a larger container with enough water to ensure that mouthparts were kept damp whilst the resin set (~3–5 h).

Video camera placement and radio-tracking array

One colour low-lux video camera (SciElex, Tasmania) continuously recorded behaviour. The camera was attached to a large aluminium tripod above the experimental enclosure (Fig. 1) and was connected to a Personal Video Recorder (AV400, Archos, China) powered by a 12-V deep-cycle battery.

Two remote radio-tracking stations consisting of a data logger/receiver combination (DCII Model D5041 and R4100 respectively, ATS) continuously recorded radio-signal strength. One station was coupled to a three-element yagi antenna (ATS) and the second station was coupled to a wand (modified gap-loop) antenna (Titley Electronics, Ballina, Australia) (Fig. 1). The yagi antenna was mounted on two wooden posts 1 m above the ground and 20 m from the artificial enclosure, with the antenna facing the enclosure. The wand antenna was mounted 2 m above the enclosure (Fig. 1). Data loggers were programmed to record radio-signal strength every 5 s. A single individual was placed in the experimental enclosure at a time; thus the period of data collection for each individual was defined as an experiment. During each experiment the signal strength of a control radio-tag (fixed to the bottom of the enclosure) was recorded on alternate 5-s periods for the duration of the study, resulting in one record of signal strength every 10 s for each tag. Video monitoring and radio-telemetry stations were time-calibrated before commencement of the study. To encourage activity of individuals during the study, canister filters (Fluval 4, Hagen Deutschland GmbH, Holm, Germany) generated a water current for alternate 15-min periods for the duration of each experiment (3 h).

A behavioural dataset was generated for each individual by post-processing video data. Trout exhibited three distinct behaviours: resting, holding position and constant movement; however, crayfish exhibited only resting or constant movement behaviours. Resting was defined as any lack of movement (apart from operculum movement in the case of trout) and was recorded when an individual was stationary. Holding position was a category applied to trout only and was defined as active fin or muscular movement to maintain position against flow. Constant swimming (or constant movement for crayfish) was

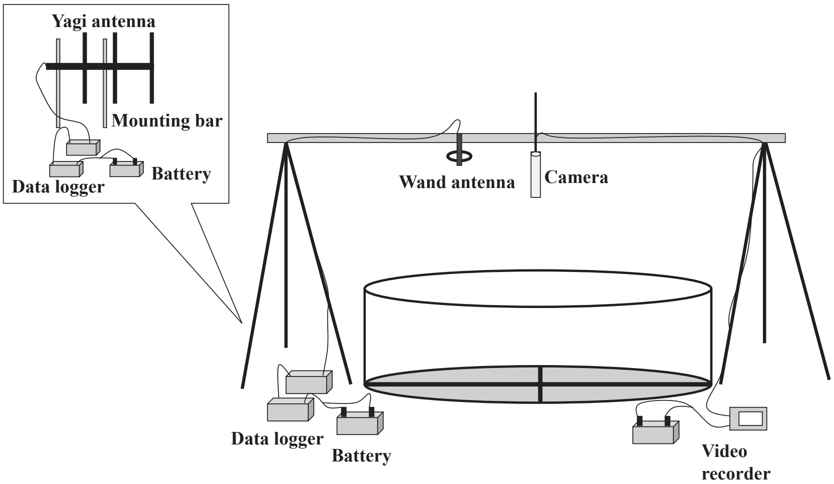

Fig. 1. Apparatus used to monitor activity of rainbow trout and Murray River crayfish.

associated with slow, continual movement resulting in a change in location within the enclosure.

Data analysis

An activity dataset was generated by calculating the standard deviation (s.d.) of three consecutive data-logged records (one record every 10 s) of radio-signal strength. This equated to one activity record every 30 s. Each 30-s activity value was subsequently allocated the predominant behavioural category observed for that 30 s. Non-parametric Kruskal–Wallis tests were used to test for differences between activity values of each behavioural category (including control) for each method, as data did not conform to parametric analysis of variance's (ANOVA) assumptions of normality, even after transformation. A *post hoc* comparison of mean ranks was also conducted. One-way ANOVA was used to compare wind speed ($m\,s^{-1}$) during the three experiments, following a Bartlett's test for homogeneity of variance and a Wilk–Shapiro normality test. *Post hoc* analysis was carried out using Tukey's Honestly Significant Difference test. All statistical analyses were conducted in Statistix for Windows (ver. 8.1), with the significance level for hypothesis tests at $P = 0.05$.

Results

Different behaviours could be determined using variation in radio-signal strength in this study (Fig. 2). This result was consistent for two taxa (fish and crayfish) exhibiting different modes of movement, the same taxa with different types of radio-tags (a whip and a coil radio-tag) and different receiving antennas (a yagi and wand antenna) monitoring the same type of radio-tag (Fig. 2).

The magnitude of activity (variation in signal strength) also reflected different types of behaviours (Fig. 2). For example, a significant difference in the magnitude of activity occurred between behaviours in the trout whip tag experiment for both the yagi (Kruskal–Wallis statistic = 220.9640, $P = 0.000$) (Fig. 2*a*) and wand (Kruskal–Wallis statistic = 421.9895, $P = 0.000$) (Fig. 2*b*) receiving antennas, in the trout coil tag experiment for both the yagi (Kruskal–Wallis statistic = 39.5516, $P = 0.000$) (Fig. 2*c*) and wand (Kruskal–Wallis statistic = 430.2803, $P = 0.000$) (Fig. 2*d*) receiving antenna, and in the crayfish coil tag experiment for both the yagi (Kruskal–Wallis statistic = 142.7604, $P = 0.000$) (Fig. 2*e*) and wand (Kruskal–Wallis statistic = 179.7374, $P = 0.000$) (Fig. 2*f*) receiving antennas.

Differentiating behaviour based on the use of variation in radio-signal strength was variable between and within treatments (Table 1). In the trout whip tag experiment, monitoring with a yagi antenna enabled differentiation of all three behaviours from each other, and the control tag. However, simultaneous monitoring with a wand antenna grouped holding position and constant movement together. In the trout coil experiment, monitoring with a yagi antenna grouped stationary and holding position together, with constant movement grouped apart from these. However, the results of this treatment were confounded by high signal strength variation from the control tag (Fig. 2*c*). In comparison, monitoring with a wand antenna grouped holding and constant movement together, with stationary and the control tag grouped out separately (Table 1). In the crayfish experiment only two behaviours were observed, stationary and constant movement (Fig. 2*e*,*f*). Monitoring by both yagi and wand antennas grouped these two behaviours separately, with stationary (no movement) and control tags grouping together in both cases (Table 1).

High levels of variation in signal strength were recorded from the control tag via a yagi antenna during the trout coil experiment as a direct result of strong wind causing the yagi to vibrate. Analysis of wind speed data recorded at 15-min intervals from a nearby weather station identified significantly higher wind speeds (mean ± s.e.) during the trout coil experiment ($2.70 \pm 0.23\ m\,s^{-1}$) in comparison with the trout whip ($1.11 \pm 0.20\ m\,s^{-1}$) and crayfish coil experiments ($1.22 \pm 0.20\ m\,s^{-1}$) (one-way ANOVA: $F_{2,36} = 17.86$, $P = 0.0000$). Simultaneous monitoring by a wand antenna was not affected by this problem due to the differences in physical dimensions of the two antennae and mounting arrangements (Fig. 1).

Discussion

This study confirms that active and inactive periods can be effectively determined for a fish and a crayfish exhibiting

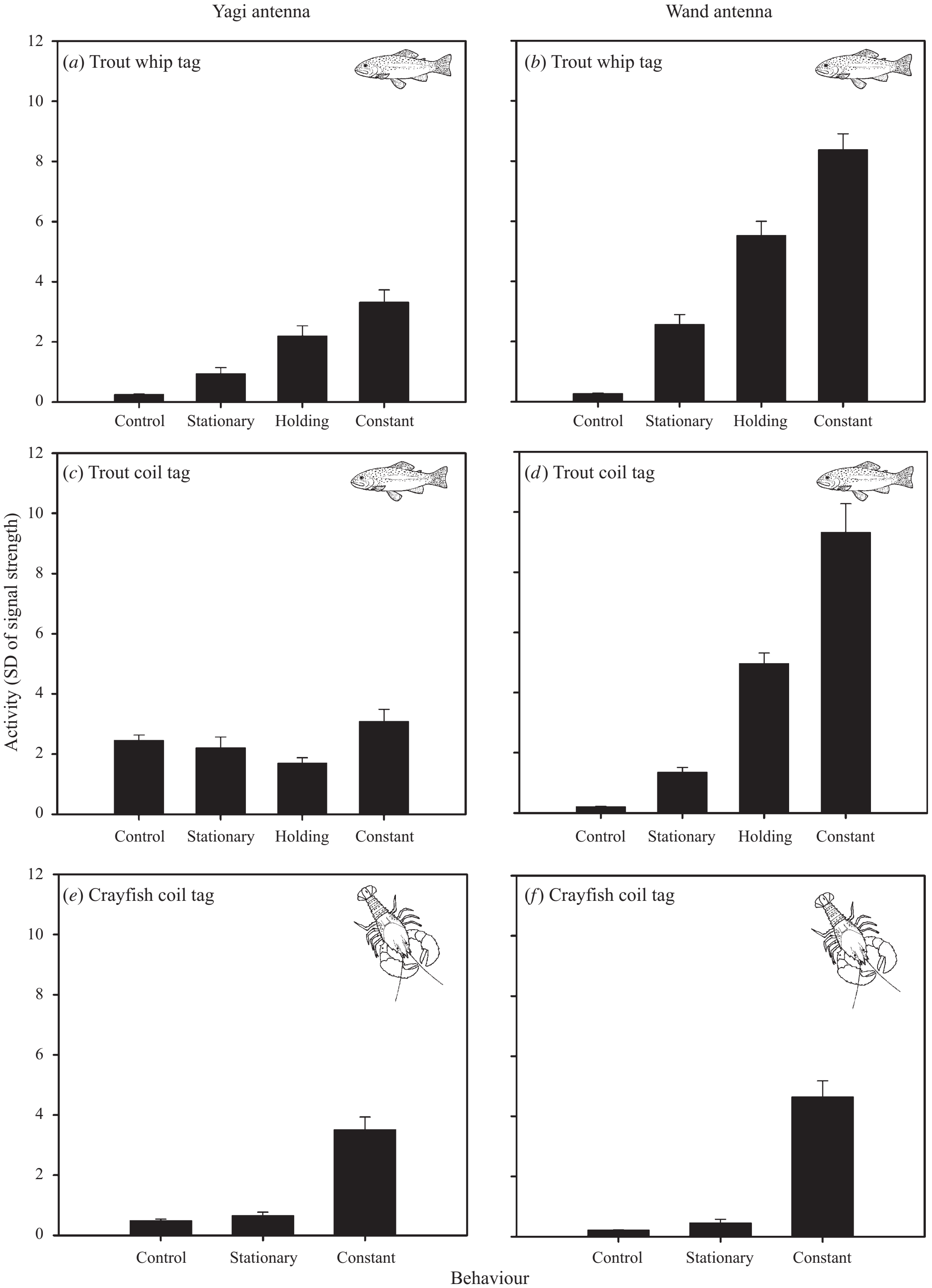

Fig. 2. Comparison of activity, using variation in radio-signal strength, separated by behavioural data collected using a video recorder. Two experiments monitored radio-tagged rainbow trout (one fitted with a whip tag and one fitted with a coil tag) and one monitored a Murray River crayfish (fitted with a coil tag). All three experiments simultaneously monitored tagged individuals with a yagi antenna and a wand receiving antenna. Data are presented as mean ± s.e.

markedly different modes of locomotion, from records of variation in radio-signal strength. While this method has previously been used to infer a relationship between signal variation and activity, subsequently reporting activity patterns of numerous fish species (e.g. David and Closs 2003; Thiem *et al.* 2008), the current study provides the first confirmation of the

Table 1. Homogenous groups based on comparison of mean ranks following Kruskal–Wallis one-way analysis of variance tests for differences between activity values of each behavioural category

Treatment	Antenna	Behaviour			
		Control	Stationary	Holding	Constant
Trout whip	Yagi	I	II	III	IV
	Wand	I	II	III	III
Trout coil	Yagi	II	I	I	III
	Wand	I	II	III	III
Crayfish coil	Yagi	I	I	–	II
	Wand	I	I	–	II

method based on a controlled experiment using live animals. Further, different levels of activity (reflecting different behaviours) were also distinguishable from study of trout. For instance, we observed that a fish does not need to change location (i.e. actively holding position in the current) to be recorded as active by this technique. If radio-signal strength is to be used as a surrogate for fish activity it will be important to carefully define active and inactive for the purposes of any particular study. For instance, distinguishing between swimming slowly to hold position as opposed to resting on the benthos, may be of limited value in a home-range study but of major relevance in a physiological study.

In the current study the minimum temporal resolution of activity was 30 s (i.e. s.d. of three 10-s records). This determined the minimum bout of any motion or behaviour that we could detect via radio-telemetry. Beaumont *et al*. (2002) demonstrated the application of an activity tag, whereby behaviours that occurred for <5 s could be recorded. However, there is often a trade-off between sample size and temporal resolution of sampling. For example, Løkkeberg *et al*. (2002) found that tracking multiple cod (*Gadus morhua*) rather than a single individual resulted in 30–70% reductions in swimming speed estimates, depending on sample sizes. Alternatively, for studies aiming to determine diel activity patterns, a 10-min resolution may be more appropriate (e.g. Thiem *et al*. 2008) and could be used while monitoring multiple individuals from a single station.

Species-specific movement characteristics also need incorporation into study design. In this study a captive crayfish was stationary for more than an hour and field studies have revealed that crayfish can remain dormant for weeks (e.g. Ryan *et al*. 2008). Clearly, the continuous monitoring capability of remote radio-telemetry has potential for studying patterns of dormancy. The spatial behaviour of the study species also needs to be matched by adequate spatial monitoring. For instance, single antenna-logger units have been used to monitor activity of fish that occupy small home-ranges (e.g. David and Closs 2001; Thiem *et al*. 2008) and multiple remote loggers or arrays can be used to record long-distance movement whilst providing continual telemetric coverage (cf. Cooke *et al*. 2004; Hanson *et al*. 2007).

In the current study different behaviours were unable to be distinguished for trout using a combination of a yagi receiving antenna and coil tag. This result is likely a consequence of high wind speeds recorded during the experiment, reflected by fluctuations in radio-signal strength of a control tag. However, as the same combination of fish, radio-tag and receiving antenna was not repeated in low wind conditions the anomaly cannot be confirmed. While it is documented that local environmental (e.g. David and Closs 2001) and habitat (e.g. Broadhurst and Ebner 2007) conditions can affect radio-signals in streams, environmental effects on the receiving capabilities of radio-telemetry equipment are poorly understood. Recent modelling of acoustic signal attenuation and receiver efficiency under different sea state conditions (A. J. Hobday and D. Pincock, unpubl. data) may provide a useful framework for testing abiotic effects on radio-signal strength. Practical solutions to minimising wind effects include firmly securing (e.g. use of heavy-duty rather than light-weight yagi antennae, use of dipole rather than directional antennae, multiple fixing points for antennae) and positioning antennae effectively (placing antennae in sheltered areas or by shielding antennae from the wind). Remote logging of wind speed and direction in addition to monitoring control radio-tags at study sites is also recommended for alerting researchers to periods when radio-signal data are likely to be poor indicators of the behaviour of radio-tagged animals. It might also be possible to use these data to calibrate radio-signal strength data collected from radio-tagged animals to overcome the problem of wind effects.

In conclusion, we have validated that variation in radio-signal strength can be used to distinguish activity from inactivity in two aquatic taxa that exhibit functionally different modes of locomotion. The validation underpins field-based applications of the technique (David and Closs 2001; Cooke *et al*. 2002; Robertson *et al*. 2004; Thiem *et al*. 2008). We recommend using control tags in field studies to overcome confounding effects from extrinsic factors, including wind. Yagi antennas will enable monitoring of activity over relatively large areas in contrast to wand antennas, and multiple yagi-logger stations will facilitate recording of fish with large home ranges. To this end, further opportunities lie with applications of multiple antenna configurations and simultaneous monitoring of multiple species.

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