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Use of an acoustic telemetry array for fine scale fish behaviour assessment of captive Paiche, *Arapaima gigas*, breeders

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Abstract

As *Arapaima gigas* is one of the most valuable species for the growing production of Amazonian aquaculture, knowledge of its reproductive behaviour and its application to increase reproduction success in captivity is of great importance as no hormonal spawning induction technique exists for this species. An acoustic positioning system (LOTEK Inc.) was used to observe the interactions of adult fish to better understand the formation of mating pairs. Fish were placed in a 4,500 m^2 aquaculture pond over a 6-month period in the IIAP field station of Pucallpa, Perú. This paper describes the methodological protocols used to set up and test the hydrophone array and presents the methodology used for the analysis of the huge amount of collected data. This methodology is illustrated by the analysis of a 6-day period for a mating pair that showed a spawning event. The results indicated that male and female occupied mostly one preferential area in one pond edge where the nesting area is located. Different activity patterns were observed during the spawning event, with male and female being closer during the spawning day. The results also showed that male travelled less distance than female during the studied period. Finally these results demonstrated the suitability of such equipment to monitor fish interactions at fine spatial (sub meter) and temporal (5 s) scales in confined environments like aquaculture ponds.

KEYWORDS
accoustic telemetry, *Arapaima*, fine scale positioning, paiche, reproductive behaviour

1 | INTRODUCTION

Arapaima gigas is an air-breathing, giant fish of the Amazon basin and the largest scaled freshwater fish in the neotropics with over 3.5 m and 250 kg. In spite of the tremendous economic and cultural importance of this species in the Amazon ragion, behavioural studies of this species are scarce. As *A. gigas* has been over-exploited for decades (Castello, Arantes, McGrath, Stewart, & Sousa, 2015), the natural populations are now seriously depleted and the species is listed in CITES Appendix II as an endangered species (CITES, 2018) and IUCN Red List of Threatened Species as a data deficient species (IUCN, 2017). This situation has led to introduce in many areas *A. gigas* into lagoons and ponds for aquaculture purposes to face the decline of wild populations and it was hoped that it would adapt to pond culture environment. One of the first difficulties encountered was the low reproduction rate in captivity as there was no artificial propagation technique for this species because of its complex behaviour and anatomical characteristics of the reproductive system

(Chu-Koo et al., 2009). Fry production relies only on spontaneous reproduction in earthen ponds after one or more pairs have formed. Under these conditions, the current production of fry remains low because of the few number of pairs formed in captivity. The purpose of our work is to better understand male–female interactions in captive pairs to improve reproductive success. Direct observation or video recordings are not possible in this earthen pond environment, the transparency of which rarely exceeds 20 cm, so we looked for other indirect means, like ultrasonic telemetry, that nevertheless allowed a precise spatiotemporal follow-up of several individuals at the same time.

Remote tracking of mobile aquatic animals in water bodies with limited visibility is the best technique to study individual movements or behaviour, social interactions and territory extension of each individual or group using generally the home range proxy (Vokoun, 2003; Worton, 1989). It is critical to know the locations of individuals over a significant period of time with the greatest possible accuracy when studying interactions between individuals, habitat preferences or reproductive behaviour. Such studies are generally based on radio telemetry (Baras, 1998; Koehn et al., 2009; Núñez-Rodríguez et al., 2015; Økland, Thorstad, Hay, Næsje, & Chanda, 2005). With manual positioning, animal detection is performed with the aid of a receiver and a "Yagi"-type antenna in the case of radio transmitters or with a hydrophone operated from a boat. Once located, coordinates of a transmitter are registered using a GPS receiver. With automatic positioning systems, the detection of transmitters is performed automatically with a few antennas or hydrophones strategically positioned and connected to data loggers, which record time and transmitter code detected the by antenna or hydrophone. The manual methods are used mainly when continuous monitoring and high accuracy or resolution of the position of more than one individual at a time is not required.

In studies that require precise positioning (at meter range or less) an array of several transmitters is necessary to obtain a coarse detection network associated with calculation systems by triangulation. In aquatic environments, this can only be done with a system of ultrasonic coded transmitters and a network of hydrophones.

Technical and miniaturization improvements have permitted the development of ultrasonic telemetry equipment in aquatic environments (Crossin et al., 2017; Hussey et al., 2015) or GPS system in aerial or terrestrial studies, which allow a much higher positioning frequency (Abecasis, Bentes, Lino, Santos, & Erzini, 2013; Bellquist, Lowe, & Caselle, 2008; Binder, Holbrook, Hayden, & Krueger, 2016; Binder et al., 2017; Espinoza, Farrugia, Webber, Smith, & Lowe, 2011; Espinoza, Heupel, Tobin, & Simpfendorfer, 2016; Farmer, Ault, Smith, & Franklin, 2013; Lowe, Topping, Cartamil, & Papastamatiou, 2003; Martins et al., 2014; Mason & Lowe, 2010; Pursche, Suthers, & Taylor, 2013; Semmens, 2008; Simpfendorfer, Heupel, & Hueter, 2002; Topping, Lowe, & Caselle, 2006; Villegas-Ríos et al., 2013).

In aquatic environments this technology is based on sound speed propagation in water (approx. 1,500 m/s at 25°C) where 1 m corresponds to 0.6 millisecond transmission delay.

At least three hydrophones are necessary to process the signal by an hyperbolic triangulation routine (Bégout & Lagardère, 1995). The best accuracy is obtained when the transmitter is equidistant from three hydrophones. Due to these characteristics, the design of the hydrophone network will depend mainly on the size and shape of the water body in which the fish movements are studied. Reception strength will also depend on some parameters such as soil type and vegetation, water stratification and acoustic noise that can generate reverberations of ultrasonic waves (Pincock & Johnston, 2012). Distance between hydrophones will depend on the hydrophone characteristics and the power of the transmitters used. An approximate range of 100–200 m to several kilometres depending on the chosen systems and topography of the water body is generally admitted.

This type of fine scale monitoring is expected to generate information, at detailed spatial and temporal ranges, on simultaneous behaviour of all fish in the pond and their interactions (Baktoft et al., 2015; Cooke et al., 2013; Pincock, Welch, McKinley, & Jackson, 2010). This paper describes the methodology used and the positioning accuracy within the hydrophones array as well as some data on a specific pair as an example of the suitability of this technique for monitoring fish behaviour in such environments. We expect that the information collected with this system will allow a better understanding of *A. gigas* reproductive ethology, which can contribute to improve management of captive breeding stocks.

2 | MATERIALS AND METHODS

2.1 | Study area

All individuals were placed together the same day in a sub-square 4,500 m^2 and 1 m average depth aquaculture earthen pond at the IIAP research station of Pucallpa, Peru (Figure 1). These ponds represent the typical breeding environments used by local fish farmers to manage *A. gigas* reproduction in captivity.

2.2 | Fish

Twenty adult *A. gigas* specimens, 10 females and 10 males of 5–6 year-old, born in captivity were selected for this study and gender was determined using the specific *Arapaima* sexing technique (Chu-Koo et al., 2009). At the same time each fish was permanently identified by an 11 mm Pit Tag injected with an appropriate syringe in the medio-anterodorsal musculature for posterior identification. All fish used were sexually mature and size and weight ranged from 144 to 188 cm TL and 26 to 65 kg respectively.

2.3 | Fish transmitter implantation

Transmitters (16 × 80 mm, 76 KHz) weighing 35 g (MM-M- 16-50-PM, LOTEK Inc.) were attached externally according to the technique described previously for radio transmitters of similar size and weight (Núñez-Rodríguez et al., 2015). Briefly, a stainless steel

FIGURE 1 Aerial view (Google © DigitalGlobe 2016) of the zone where the experimental pond (8°24′02″S–74°38′26″W) is situated and positions of the eight hydrophones (numbered circles) forming the array inside the pond [Colour figure can be viewed at wileyonlinelibrary.com]

plastic coated cable was inserted using an adapted needle near the dorsal fin after local anaesthesia with 0.5% lidocaine (0.5 ml on each side), and transmitter were fixed to the cable with plastic clamps. The needles and cables were previously sterilized with 70% ethanol and an anti-bacterial solution (10% Povidone) was applied on the injection sites. The whole tagging procedure lasted less than 2 min. The manipulation did not alter fish behaviour notably since fish recovered a normal quiet swimming immediately after their release in the experimental pond.

2.4 | Hydrophones array geometry

Eight hydrophones (WHS 3250D, LOTEK Inc.) have been immersed at the periphery of the 4500 m^2 square-shaped pond (Figure 1), fixed on metal poles (3 cm in diameter, 2 m long) placed approximately every 25 m and 1 m away from the shoreline. The average depth of the pond varied from 0.8 m at the shoreline to 1.20 m in the central area with a relatively smooth muddy bottom. Hydrophone antennas were placed approximately 50 cm above the bottom of the pond and 30–50 cm from the water surface depending on water depth at each location. The exact coordinates of each hydrophone and pond-shoreline were determined using a sub meter GPS (Trimble GeoExplorer 6000 XH 3.5G) giving a position accuracy of approximately 30 cm according to manufacturer's indications.

3 | RESULTS

All 20 adult fish implanted with acoustic transmitters survived and were tracked successfully for a 6 month-period, 24 hr a day. Data were downloaded from the eight receivers located at the periphery of the pond (Figure 1) on a weekly basis. In average, from a maximum possible number of 17,280 bearings per individual and per day corresponding to one detection every 5 s which was the pulse interval of transmitters, 11,500 ± 2,800 bearings were recorded, representing a detection efficiency of 66.55%. Over the 6 months monitoring, an average of 2,070,000 ± 504,000 bearings were recorded for each individual. The data processing methodology is described hereafter to illustrate the behavioural assessment of the single mating pair that showed a spawning event during this period (data correspond to a 6-day period from which only 2.5 days were analysed in detail).

3.1 | Array accuracy

A single transmitter positioned approximately in the centre of the pond, which is theoretically the best detection position by the array (Figure 2), was used to test the precision of the positions calculated by the U-Map package. The transmitter was maintained 50 cm above the bottom of the pond and approximately 40 cm from the water surface. Bearings calculations were possible only when at least three hydrophones received the tag signal. The positions were recorded every minute for 1,378 successive positions (Figure 3). The results showed that bearings were not exactly randomly distributed around the mean position of the transmitter as standard deviations on both axes, calculated on average position, were slightly different (*x* axis ± 0.65 cm *SD*; *y* axis ± 0.56 cm *SD*). A preferential distribution in the W-SW to E-NE direction was observed (r^2 = .13, n = 1378).

3.2 | Detection efficiency

Detection of hydrophone array efficiency was analysed using Qgis 2.12 package (QGIS Development Team, 2016. QGIS Geographic

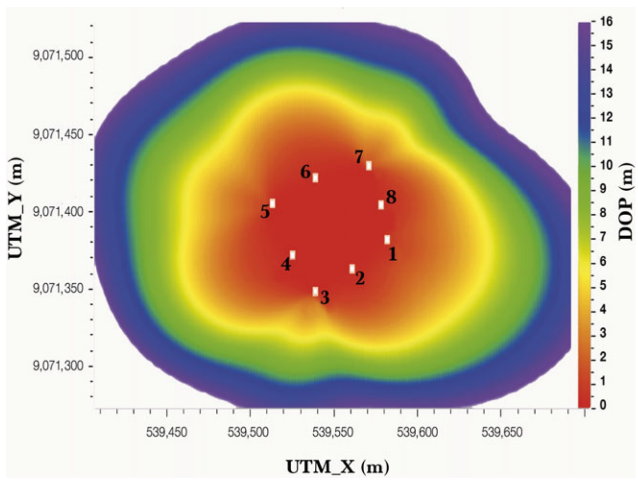

FIGURE 2 Dilution of precision (DOP) of the hydrophone array as calculated by Lotek's U-Map software (right axis). Hydrophone positions are given in UTM (Universal Transverse Mercator) system (in meters). Numbers correspond to the location of the eight hydrophones of the array. Pond limit is indicated by a solid line [Colour figure can be viewed at wileyonlinelibrary.com]

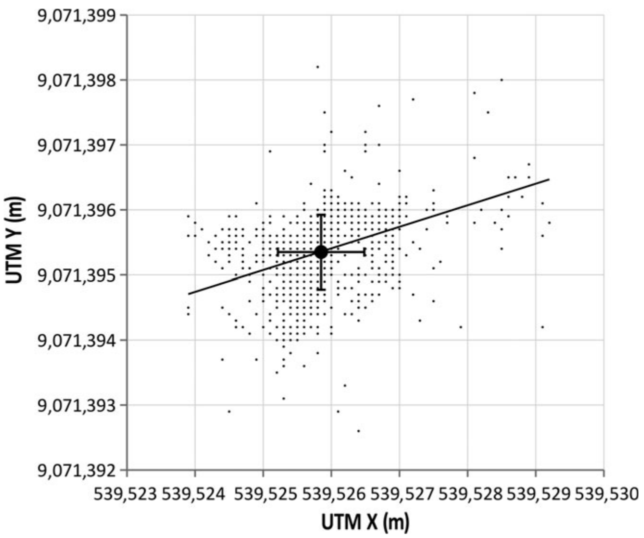

FIGURE 3 Precision of acoustic telemetry array. Data represent the coordinates recorded every minute (n = 1,378) of a single transmitter (not all points are visible due to overlapping) positioned approximately at the middle of the pond, 50 cm above the bottom. Solid black circle corresponds to the average position (± standard deviation error bars) and black line represents the linear regression over all bearings ($y = 0.33X + 8.89.$ 10E6, $R^2 = .14$). Axes values are expressed in meters using UTM system.

Information System. Open Source Geospatial Foundation Project. http://www.qgis.org/) by plotting unfiltered bearings calculated by U-Map and then filtered by pond shape. All bearings falling out of the pond area were discarded (Figure 4). Another filter was then applied to eliminate successive points that were separated by a distance higher than that obtained with a displacement speed >5 km/hr which was the maximum average speed observed in previous tests. During this 2.5-day monitoring period, from a total of 38,558 bearings recorded, 6.4% (pond shape filter) and 1.1% (speed filter) of the calculated positions were discarded for this male. Similar procedure applied to six other fish bearings allowed us to calculate that recorded bearings represented more than 90% of real positions (90.3 ± 2.8%). Please note that due to overlapping specially in the pond area all points are not visible.

3.3 | Space occupation and activity

Interaction of male and female fish was first determined using the home range methodology. The filtered data by pond shape and speed filter (Figure 5a,b) were processed using a R macro to set a time-synchronized data sequence for male and female bearings and then formatted for OpenJump import filter. The fixed time here between two successive positions was set at 30 s in order to lower the number of data for each calculation run. Home range calculations (Figure 5c,d) have been performed using Horae (Steiniger & Hunter, 2012) package for OpenJump (Steiniger & Bocher, 2009). The data reduction process (one bearing per 30 s) did not significantly alter the accuracy (results not shown). In Figure 5c,d grey shaded areas represent the Kernel density estimation (KDE) at 95% of probability. This means that 95% of the bearings fall into the calculated home range area. In this particular case the two fish (male and female) were mostly present only in one edge of the pond, the female showing a higher displacement activity than the male. The same technique was applied for 50% and 10% probabilities of presence (Figure 6). Both fish spent most of their time on two specific areas in one edge of the pond where two nests were detected. But in only one occasion during the 2.5 day period, male and female travelled simultaneously all around half of the pond area very closely (Video S1) and then returned to the nesting zone (Figure 7). We do not know if spawning took place before or after this particular behavior observed only once during this survey. The straight travelling line for the female corresponded to interpolated trajectories for the time where no detections were received by hydrophones. The travelled distance during the 6-day period was higher for female (22,813 m) than for male (12,804 m) indicating that male spent more time around the nesting area than the female (Figure 7).

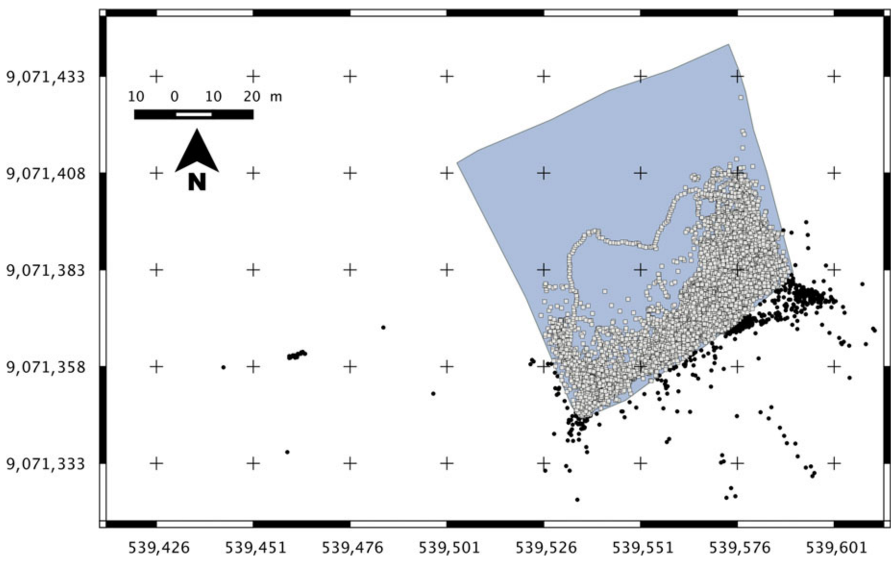

FIGURE 4 Detection efficiency of the 8-hydrophone array. Coordinates are expressed in meters using the UTM system. Data correspond to the bearings of a male *Arapaima gigas*. Black dots represent unfiltered bearings (n = 30,558); grey squares represent filtered bearings (n = 28,603) by pond shape (blue background). Out of pond points (n = 1,955) represented 6.4% of total bearings. Not all points are visible due to overlapping [Colour figure can be viewed at wileyonlinelibrary.com]

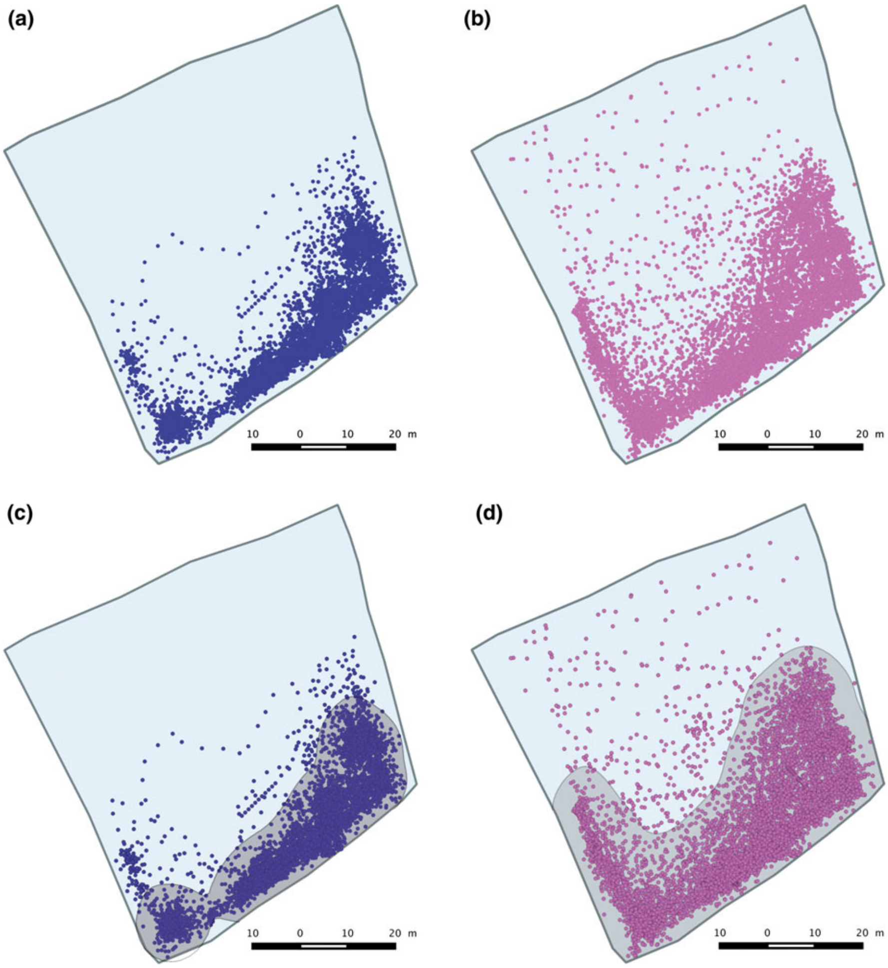

FIGURE 5 Space occupation of a mating pair of *Arapaima gigas* in the pond (2.5 days of survey). Points represent calculated positions every 30 s. (a) Male bearings (blue dots); (b) Female bearings (purple dots). (c, d) grey shaded area corresponds to 95% KDE (Kernel Density estimation) of male (c) and female (d) [Colour figure can be viewed at wileyonlinelibrary.com]

The distance between both fish was calculated during a 6-day period including the 2.5 days of detailed survey where the spawning day occurred. Male–female distance varied from less than a meter to 45 m (results not shown), but the mean daily distance reached a minimum of 4.71 ± 0.15 m *SE* the day of spawning (Figure 8). During the three previous days the distance progressively decreased significantly ($p < .01$) from 13.14 ± 0.34 m *SEM* to 4.71 ± 0.15 m *SEM* and then significantly ($p < .01$) increased during the following days to reach 14.82 ± 0.33 m *SEM* and 13.14 ± 0.42 m *SEM* on day 1 and 2 respectively (Figure 8).

4 | DISCUSSION

4.1 | Accuracy

Array accuracy has been determined using a fixed transmitter at known coordinates in the geometric centre of the detectors array. As described in another study (James, Fischer, Laube, & Spindler, 2014) we were expecting an homogeneous distribution on all directions as the test transmitter was placed approximately at the centre of the array. Nevertheless as previously mentioned (Bergé et al., 2012; Binder et al., 2016) this finding showing an oriented distribution could be attributed to sound wave reflection on the pond shoreline, which slope is not perfectly similar on all its length. But if we consider the observed accuracy (<1 m) we still have highly reliable position accuracy as fish average size was 1.67 ± 0.15 m. This accuracy around the meter range is similar to that obtained with Chinook salmon in similar 4,000 m^2 enclosure using a 9-hydrophone array (Semmens, 2008) and an 8-hydrophone array in a 10,000 m^2 natural lake (Baktoft et al., 2015). Although this technique gives the best accuracy over all other telemetry techniques in turbid environment, the underwater video observation would be of great interest to get complementary and detailed data on fish behaviour as reported for other aquatic animals (Ebner, Clear, Godschalx, & Beitzel, 2009; Fatsini, Rey, Ibarra-Zatarain, Mackenzie, & Duncan, 2017; Mills, Verdouw, & Frusher, 2005; Struthers, Danylchuk, Wilson, & Cooke, 2015). Nevertheless in turbid environments the video option is not possible.

4.2 | Detection efficiency

Theoretical detection distance (few-hundred meters) was virtually higher than the largest possible distance (diagonal) of the pond used in this study, which corresponded to 102 m. Nevertheless the effective average detection rate reached only 66.55%. The reason, as mentioned in other studies (Binder et al., 2016; Hartill,

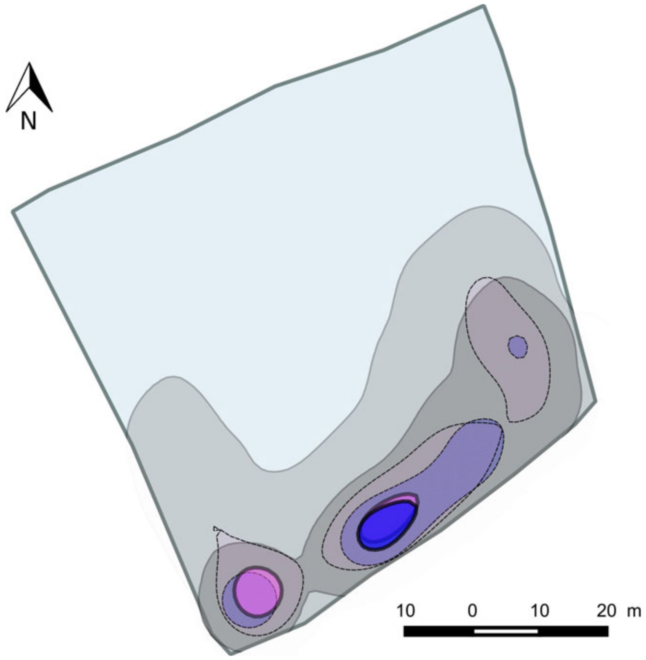

FIGURE 6 Home range areas of male and female *Arapaima gigas* using KDE at 95% (light grey: female; dark grey: male), KDE50% (doted line shaded purple: female; shaded blue: male) and KDE10% (solid purple: female; solid blue: male). Each position is calculated every 30 s for a 2.5 days period [Colour figure can be viewed at wileyonlinelibrary.com]

Morrison, Smith, Boubée, & Parsons, 2003), is probably due to fish orientation or position close to the shore inducing signal absorption, background noise or signal collisions when numerous transmitters are present that did not allow a valid calculation position by the LOTEK's U-Map software. A small percentage of detections corresponded to out of pond bearings (6.4%) and some in-pond wrong bearings too (1.1%) identified by the speed filter. Finally the corrected detection rate reached around 50%, which means a valid detection every 10 s in average which is of the same order of magnitude reported for 0.01 km^2 natural lake with similar equipment using an array of eight hydrophones (Baktoft et al., 2015). Nevertheless this detection rate allowed to monitor fish movements and interactions quite precisely as showed by the male–female interaction during a spawning event.

4.3 | Space occupation and activity

Fish were most of the time close to the shore probably because of nest building and cleaning activity in the pre-spawning period as the male and female observed showed a spawning event during the studied period and generally nests are built in the vicinity of the shore area where the water depth is smaller (Bard & Imbiriba, 1986; Imbiriba, 1991). The short distance between male and female might be a good indicator of mating behaviour and proximity of a spawning event. In this pair, the lower distance between male and female and the duration of the period when both fish were closer coincided with an effective spawning. We will apply this parameter to all fish present in the pond to detect potential mating pairs during the 6 month-period of survey and look to the permanency of these pairs. In previous observations on reproductive behaviour of *A. gigas*, it was thought that only one nest was built by male and female (Bard & Imbiriba, 1986; Imbiriba, 1991) but for this particular pair

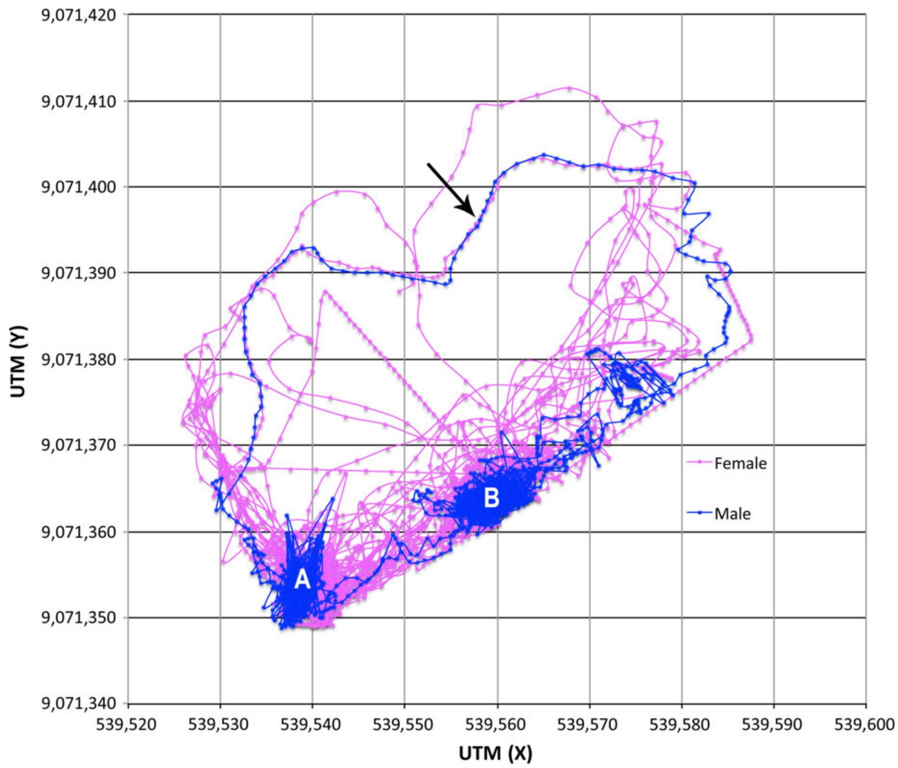

FIGURE 7 Visualization of the positions and trajectories of two paiches, Male (blue) and Female (purple) during 9.5 hr (from 0:00 to 9:30 a.m.) corresponding to the period of spawning. Each position is determined every 10 s. The arrow indicates a very peculiar trajectory where both male and female are travelling together towards the middle of the pond and then coming back to the nesting areas (here two areas labelled A and B) [Colour figure can be viewed at wileyonlinelibrary.com]

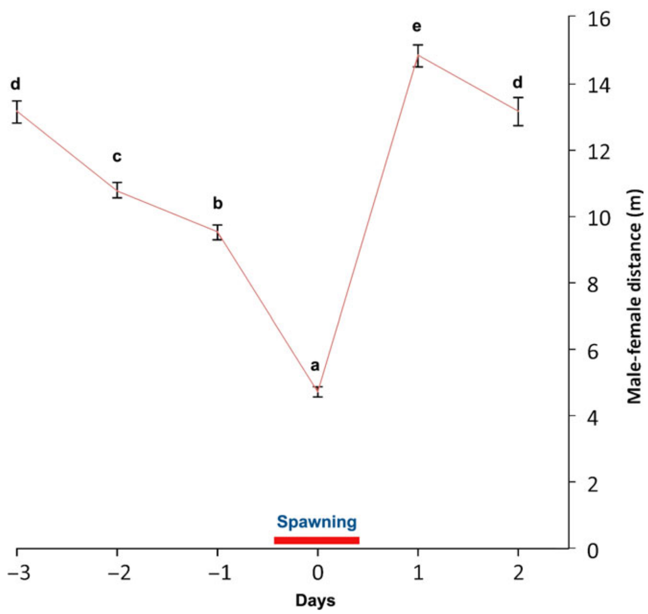

FIGURE 8 Male–Female *Arapaima gigas* proximity for a mating pair during a 6-day period (−3, −2, −1, 0, 1, 2) in the course of a spawning event (which occurred the fourth day of this follow-up, noted 0 in X graph axis and indicated by the red line). Data represent averaged distances in meters between male and female, calculated every minute, for an entire day (0:00–24:00 hr). Error bars represent standard error of the means and different superscript letters indicate significant differences between means at $p < .001$, using Tambane's T2 post hoc test for unequal variance samples [Colour figure can be viewed at wileyonlinelibrary.com]

we observed during the survey period that two different nests were built although only one was used for egg deposition.

5 | CONCLUSION

The results obtained with the acoustic telemetry array deployed in this study establish the suitability of such ultrasonic telemetry equipment to monitor, at fine spatial and temporal scales, multiple fish interactions in confined turbid environments like aquaculture ponds. The first results presented here in *A. gigas*, provides new information on fish movements, space occupation, male–female interactions and reproductive behaviour in an earthen pond. This preliminary work will be extended to the analysis of all data collected on the survey of 20 *A. gigas* individuals (10 males and 10 females) during a 6-month period.

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Additional Supporting Information may be found online in the supporting information section at the end of the article.

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