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1 **Use of an acoustic telemetry array for fine scale fish behavior assessment of captive**
2 **Paiche, *Arapaima gigas*, breeders.**

3

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20

21 **Abstract**

22 *As Arapaima gigas* is one of the most valuable species for the growing production of
23 amazonian aquaculture, knowledge of its reproductive behavior and its application to
24 increase reproduction success in captivity is of great importance. An acoustic positioning
25 system (LOTEK Inc.) was used for monitoring the movements of twenty adult fish (10
26 males and 10 females) with a meter range precision. Fish were placed in a 4 500 m² pond
27 over a six-month period in the IIAP field station of Pucallpa, Peru. This paper describes the
28 methodological protocols used to set up and test the array and presents the strategy for the
29 analysis of collected data. This methodology is illustrated by the analysis of a 2.5 days
30 period for a mating pair that showed a spawning event. The results indicate that males and
31 females occupy mostly one preferential area in one pond edge where the nesting area is
32 located. Different activity patterns were observed during the spawning event and after.
33 Males and females were much closer for long periods of time during the pre-spawning

34 period. The results also show that males travel less distance than females and that
35 movements are not correlated with day/night cycle. Finally these results demonstrate the
36 suitability of such equipment to monitor fish behavior at fine spatial and temporal scales in
37 confined environments like aquaculture ponds.

38
39

40 **Introduction**

41 Remote tracking of mobile aquatic animals in water bodies with limited visibility is the best
42 technique to study individual movements or behavior, social interactions and territory
43 extension of each individual or group using generally the home range proxy (Vokoun,
44 2003; Worton, 1989). It is critical to know the locations of individuals over a significant
45 period of time with the greatest possible accuracy when studying interactions between
46 individuals, habitat preferences or reproductive behavior. Such studies are generally based
47 on radio telemetry (Baras, 1998; Koehn et al., 2009; Núñez-Rodríguez et al., 2015; Økland,
48 Thorstad, Hay, Næsje, & Chanda, 2005). With manual positioning, animal detection is
49 performed with the aid of a receiver and a "Yagi"-type antenna in the case of radio
50 transmitters or with a hydrophone operated from a boat. Once located, coordinates of a
51 transmitter are registered using a GPS receiver. With automatic positioning systems, the
52 detection of transmitters is performed automatically with antennas or hydrophones
53 strategically positioned and connected to data loggers, which record time and transmitter
54 code detected the by antena or hydrophone. These methods are used mainly when
55 continuous monitoring and high accuracy or resolution of the position of more than one
56 individual at a time is not required.

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

61 Technical and miniaturization improvements have permitted the development of
62 ultrasonic telemetry equipment in aquatic environments or GPS system in aerial or
63 terrestrial studies, which allow a much higher positioning frequency (Abecasis, Bentes,
64 Lino, Santos, & Erzini, 2013; Bellquist, Lowe, & Caselle, 2008; Espinoza, Farrugia, Webber,
65 Smith, & Lowe, 2011; Farmer, Ault, Smith, & Franklin, 2013; Lowe, Topping, Cartamil, &

66 Papastamatiou, 2003; Martins et al., 2014; Mason & Lowe, 2010; Pursche, Suthers, &
67 Taylor, 2013; Semmens, 2008; Topping, Lowe, & Caselle, 2006; Villegas-Ríos et al., 2013).

68 In aquatic environments this technology is based on sound speed propagation in water
69 (approx. 1 500 meters per second at 25 °C) where 1 m corresponds to 0.6 millisecond.

70 At least three received signals by hydrophones are necessary to be processed by hyperbolic
71 triangulation routine. The best accuracy is obtained when the transmitter is equidistant
72 from three hydrophones. Due to these characteristics, the design of the hydrophone
73 network will depend mainly on the size and shape of the water body in which the fish
74 movements are studied. Reception strength will also depend on some parameters such as
75 soil type and vegetation, water stratification and acoustic noise that can generate
76 reverberations of ultrasonic waves (Pincock & Johnston, 2012). Distance between
77 hydrophones will depend on the hydrophone characteristics and the power of the
78 transmitters used for the study. An approximate range of 100 to 200 meters to several
79 kilometers depending on the chosen systems and topography of the water body is
80 generally admitted.

81 This type of fine scale monitoring is expected to generate information, at detailed spatial
82 and temporal ranges, on the behavior of all fish in the pond and their interactions.
83 Determining the behavioral characteristics that lead to the formation of stable mating
84 pairs, but also get more information on distances between fishes, day and night activity
85 shifts, if any, and the corresponding speeds and space travelled, could have great interest
86 for the management of reproductive stocks in order to significantly increase fry production
87 of paiche. In this paper we describe the methodology used and the positioning accuracy
88 within the array as well as some data on a specific pair as an example of the suitability of
89 this technique for monitoring fish behavior in such environments.

90 We expect that the information collected with this system will allow a better understanding
91 paiche ethology, which can contribute to improve management of captive breeding stocks.

92

93

94 **Materials and Methods**

95

96 **Fish**

97 Twenty adult *Arapaima gigas* specimens, ten females and ten males of five to six year-old,
98 where selected for this study (table I). They where previously sexed using the specific

99 *Arapaima* sexing technique (Chu-Koo et al., 2009). Fish ranged from 144 to 188 cm TL and
100 26 to 65 kg of bodyweight.

101 All individuals were placed together the same day in a sub-square 4 500 m² pond at the
102 IIAP research station of Pucallpa, Peru (Fig. 1).

103

104 Fish Marking

105 Transmitters (16x80 mm, 76 Khz) weighing 35 g (MM-M- 16-50-PM, LOTEK Inc.) were
106 attached externally according to the technique described previously for radio transmitters
107 of similar size and weight (Núñez-Rodríguez et al., 2015). Briefly, a 3 mm Ø stainless steel
108 plastic coated cable was inserted using an adapted needle in the dorsal musculature after
109 local anesthesia with lidocaine, and transmitter were fixed to the cable with plastic clamps.
110 Fish were also individually identified by 11x2 mm pit tags that were inserted with an
111 appropriate syringe on the left antero-medio dorsal part of the fish.

112

113 Hydrophones array geometry

114

115 Eight hydrophones (WHS 3250D, LOTEK Inc.) have been immersed at the periphery of the
116 4500 m² square-shaped pond (Fig. 1), fixed on metal poles (3 cm in diameter, 2 m long)
117 placed approximately every 25 m and 1 m away from the shoreline. The average depth of
118 the pond varied from 0.8 m at the shoreline to 1,20 m in the central area with a relatively
119 smooth muddy bottom. Hydrophone antennas were placed approximately 50 cm above the
120 bottom of the pond and 30-50 cm from the water surface depending on water depth at each
121 location. The exact coordinates of each hydrophone and pond-shoreline were determined
122 using a sub meter GPS (Trimble GeoExplorer 6000 XH 3.5G) giving a position accuracy of
123 approximately 30 cm according to manufacturer's indications.

124

125

126 **Results**

127

128 All 20 adult fish implanted with acoustic transmitters (Table 1) survived and were tracked
129 successfully for a 6 month-period, 24 h hours a day. Data were downloaded from the 8
130 receivers located at the periphery of the pond (Fig. 1) on a weekly basis. In average,
131 11 500 ± 2 800 bearings per individual per day (from a maximum possible number of

132 17 280 bearings corresponding to one detection every 5 seconds which was the pulse
133 interval of transmitters) were recorded, representing a detection efficiency of 66,55 %.
134 Over the 6 months monitoring, an average of $2\,070\,000 \pm 504\,000$ bearings were recorded
135 for each individual. The data processing methodology is described hereafter to illustrate
136 the detailed behavioral assessment (data corresponding to 2.5 days of survey of a mating
137 pair which showed a spawning event during this period).

138

139 Array accuracy

140

141 Positioning a single transmitter approximately in the middle of the pond, which is
142 theoretically the best detection position by the array (Fig. 2), tested the precision of the
143 positions calculated by the U-Map package. The transmitter was maintained 50 cm above
144 the bottom of the pond and approximately 40 cm from the water surface. The real position
145 was determined with the Sub-meter GPS (precision ± 30 cm, as indicated by the shaded
146 grey circle in Fig. 3). Bearings calculations were possible only when at least three
147 hydrophones received the tag signal. The positions were calculated every minute (43
148 successive positions). The results showed that bearings were not randomly distributed
149 around the real position of the transmitter but oriented in a 45° angle axis to the left of the
150 North-South direction corresponding to an accuracy of $\pm 0,5$ m on North - South axis and
151 1,1 m on the East-West axis (Fig. 3).

152

153 Detection efficiency

154

155 Detection of hydrophone array efficiency was analyzed using Qgis 2.12 package (QGIS
156 Development Team, 2016. QGIS Geographic Information System. Open Source Geospatial
157 Foundation Project. <http://www.qgis.org/>) by plotting unfiltered bearings calculated by U-
158 Map and then filtered by pond shape. All bearings falling out of the pond area were
159 discarded. Another filter was then applied to eliminate successive points that were
160 separated by a distance higher than that obtained with a displacement speed >5 km.h⁻¹
161 which was the maximum average speed observed in previous tests. During this 2.5-days
162 male monitoring, from a total of 38 548 bearings 6,4% (pond shape filter) and 1,1% (speed
163 filter) of the calculated positions were discarded. Similar procedure applied to 6 other

164 paiche bearings allowed us to calculate that recorded bearings represented more than 90%
165 of real positions ($90.3 \pm 2,8 \%$).

166

167 Space occupation and activity

168 Interaction of male and female fish was first determined using the home range
169 methodology. The filtered data by pond shape and speed filter (Fig. 5a,b[F1]) were
170 processed using a R macro to set male and female bearings synchronization and then
171 formatted for OpenJump import filter. The fixed time here between two successive
172 positions was 30 seconds in order to lower the number of data for each calculation run.
173 Home range calculations (Fig. 5c, d) have been performed using Horae (Steiniger & Hunter,
174 2012) package for OpenJump (Steiniger & Bocher, 2009). This data reduction process did
175 not significantly alter the accuracy (results not shown). In Fig. 5c, d grey shaded areas
176 represent the Kernel density estimation (KDE) at 95% of probability. This means that 95%
177 of the bearings fall into the calculated home range areas. In this particular case the two
178 paiches (male and female) were mostly present only in one edge of the pond, the female
179 showing a higher displacement behavior than the male. The same technique was applied
180 for 50% and 10% probabilities of presence (Fig. 6). Both fish spent most of their time on
181 two specific areas in one edge of the pond where two nests were detected. But in only one
182 occasion male and female travelled simultaneously all around half of the pond surface very
183 closely and then returned to the nesting area (Fig. 7). Straight line travelling corresponded
184 to interpolated trajectories for periods where no detections were received for the male. We
185 do not know if spawning took place before or after this peculiar behavior observed only
186 one time during the 2.5 days of survey. The travelled distance during this 2.5 days period
187 was higher for female (4 933 m) than for male (3 142 m) indicating that the male spent
188 more time in the nest area than the female (Fig. 7).

189 The influence of day or night periods on male-female interactions was also investigated.
190 The distance between both fish was calculated using the same 2.5 days period (Fig. 8).
191 Male-female distance varied from less than a meter to 45 meters, with apparently no
192 correlation with day and night. But in the first day of survey (corresponding to the day of
193 spawning) fish were closer almost all the time while the second day they showed
194 larger periods where they were much more distant.

195

196 **Discussion**

197

198 Accuracy

199 Array accuracy has been determined using a fixed transmitter at known coordinates in the
200 geometric center of the detectors array. The accuracy ranged from 0.5 m on the North-
201 South and 1,1 m on the east-west directions. As described in another study (James, Fischer,
202 Laube, & Spindler, 2014) we were expecting homogeneous accuracies on both directions as
203 the test transmitter was placed approximately at the center of the geometric array.
204 Nevertheless as previously mentioned (Bergé et al., 2012) this finding could be attributed
205 to sound wave reflection on the pond shoreline, which slope is not perfectly similar on all
206 its length. But if we consider the lowest observed accuracy (1,1 m) we still have highly
207 reliable position accuracy as fish average size was $1,67 \pm 0,15$ m. This accuracy around the
208 meter range is similar to that obtained with Chinook salmon in similar 4 000 m² enclosure
209 using a 9 hydrophone array (Semmens, 2008).

210

211 Detection efficiency

212 Detection distance was virtually higher than the largest possible distance (diagonal) of the
213 pond, which corresponded to 102 m. Nevertheless the effective average detection rate
214 reached only 66,55 %. The reason, as mentioned in other studies (Hartill, Morrison, Smith,
215 Boubée, & Parsons, 2003), is probably due to fish orientation or position close to the shore
216 inducing signal absorption, background noise or signal collisions that didn't allow a valid
217 calculation position by the LOTEK's U-Map software. A small percentage of detections
218 corresponded also to out of pond bearings (6,4 %) and some in-pond wrong bearings too
219 (1,1 %) identified by the speed filter. Finally the corrected detection rate reached around
220 50%, which means a valid detection every 10 seconds in average. Nevertheless this
221 detection rate allowed to monitor fish movements and interactions quite precisely as
222 showed by the male-female interaction during a spawning event.

223

224

225 Space occupation and activity

226 We did not observe during this short period of time (2.5 days) different activity patterns
227 between night and day for both fish as in kelp bass, *Paralabrax clathratus* (Lowe et al.,
228 2003). Fish spent most of the time close to the shore probably because of nest building and
229 cleaning activity in the pre-spawning period as the male and female observed showed a

230 spawning event during this 2.5 days period and generally nest are built in the vicinity of the
231 shore area where the water depth is smaller (Bard & Imbiriba, 1986) (Imbiriba, 1991). The
232 short distance between male and female fish might be to be a good indicator of mating
233 behavior. In this particular pair, the lower distance and the duration of the period when
234 both fish are very close coincided with the vicinity of a reproductive event. We will apply
235 this parameter to all fish to detect potential mating pairs during the six month-period of
236 survey and look to the permanency of these pairs. During this 2.5 days period, the female
237 exhibited a higher displacement rate than male. This probably indicates that male
238 contributes more to the digging and cleaning of the nests than the female. In this case there
239 was at least two different nests while in previous observations on reproductive behavior of
240 paiche it was thought that only one nest was built by male and female (Bard & Imbiriba,
241 1986 ; Imbiriba, 1991).

242

243 Conclusion

244 The results obtained with the acoustic telemetry array deployed in this study have
245 demonstrated the suitability of such equipment to monitor, at fine spatial and temporal
246 scales, fish behavior in confined environments like aquaculture ponds. The first results
247 presented here in *Arapaima gigas*, provides new information on fish movements, space
248 occupation, male -female interaction and reproductive behavior in pond. This preliminary
249 work will be extended to the data collected on 20 paiches during a 6 months period of
250 survey.

251

252

253 Acknowledgements

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255 (Programa Nacional de Innovación para la Competitividad y Productividad, Innóvate-
256 Perú) and the IRD International Joint Laboratory "Evolution and Domestication of the
257 Amazonian Ichthyofauna" (LMI EDIA).

258

259

260 Table I: Characteristics of *Arapaima gigas* used for transmitters implantation.

261

Transmitter code	Gender	Std Length (cm)	Weight (kg)
55748	F	175	52
54500	F	159	42
54760	F	157	40
55072	H	152	37
55592	F	151	37
55436	F	181	56
55228	F	144	32
54552	F	154	37
54656	F	163	38
54812	F	155	48
55280	M	173	43
54708	M	184	65
55020	M	179	55
54864	M	134	26
54916	M	188	60
54968	M	182	56
55696	M	180	63
55124	M	178	52
55488	M	180	49
55384	M	175	50

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267 Figure 1. Aerial view of the experimental pond and localization of the eight hydrophones
268 array (red circles).

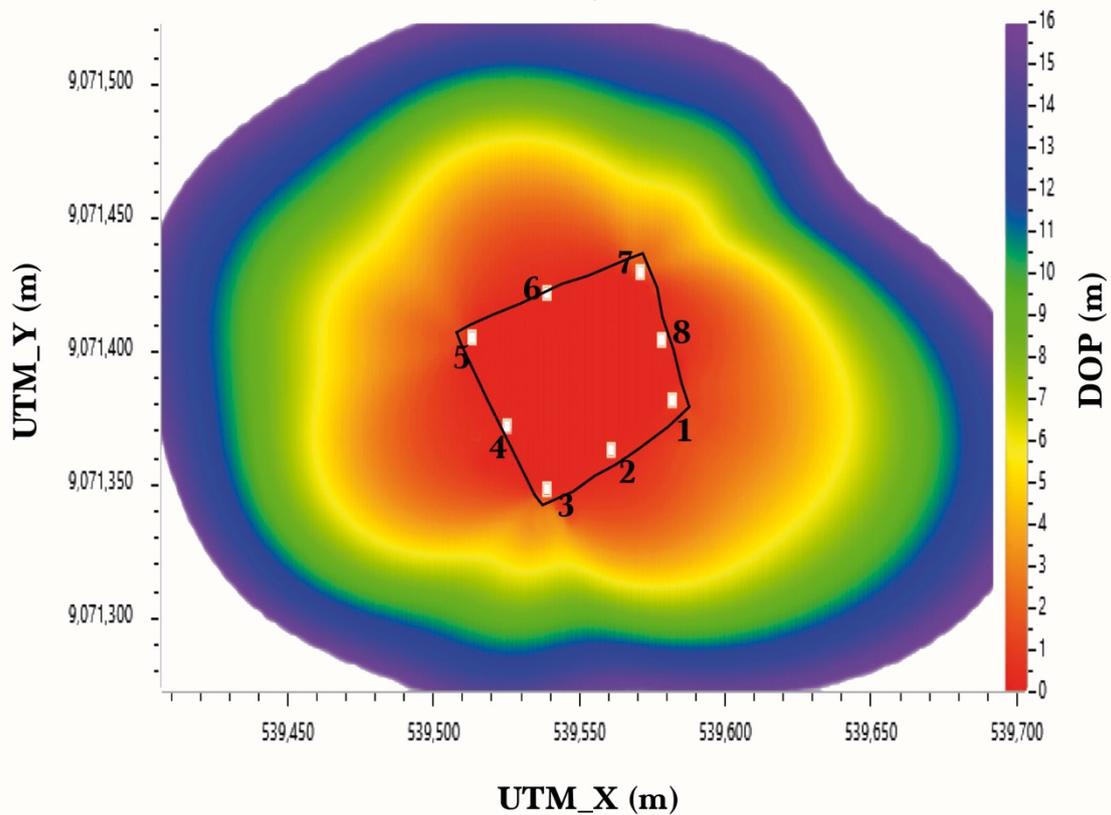
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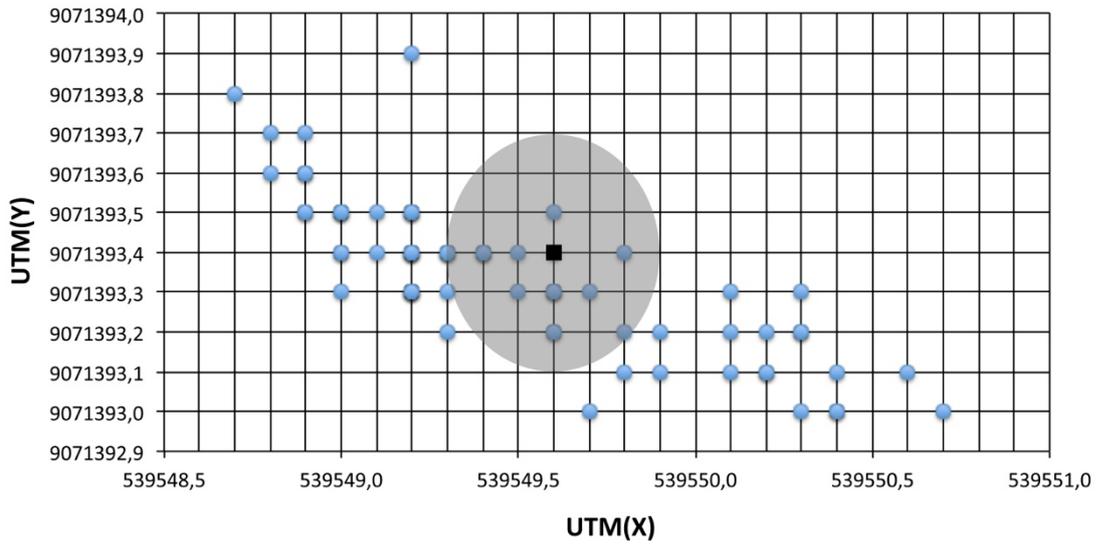
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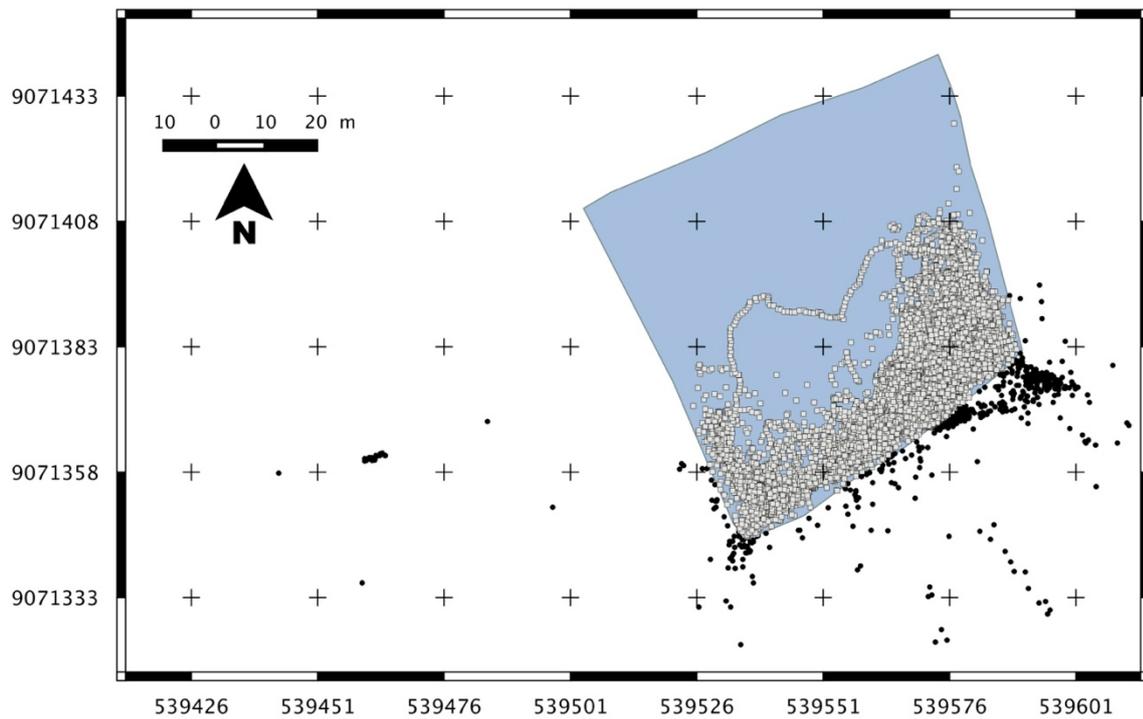
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 275
 276 Figure 2. Dilution of precision (DOP) of the hydrophone array as calculated by Lotek's U-
 277 Map software. Hydrophone coordinates are given in UTM (Universal Transverse Mercator)
 278 system (in meters). Numbers correspond to the location of each hydrophone of the array.
 279 All the pond surface (solid line) is included in the best DOP area of the array.
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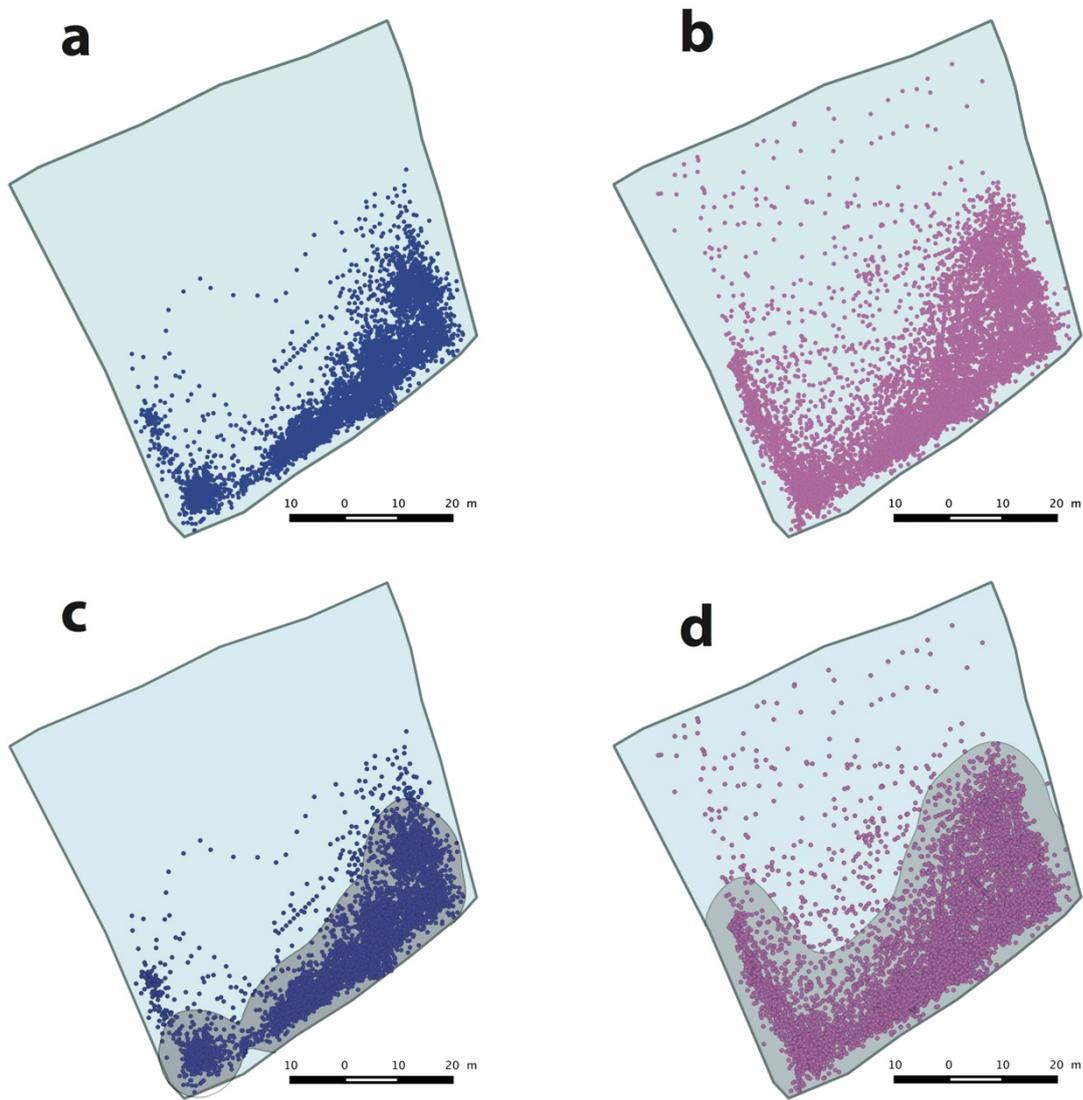
Figure 3. Precision of acoustic telemetry array. Shaded area corresponds to the GPS positioning precision of the transmitter (grey circle, corresponds to a ± 0.3 m position precision of the sub-metric GPS around the theoretical position, black square). Blue dots represent different positioning coordinates calculated every minute by LOTEK's U-Map software with the data of a single transmitter positioned approximately at the middle of the pond, 50 cm above the bottom. Axes values are expressed in meters.



298
 299 Figure 4. Detection efficiency of the hydrophone array. Unfiltered (30 558 bearings ; black
 300 dots) and filtered, (28 603 bearings ; grey squares), by pond shape (blue background).
 301 Coordinates are expressed in meters using the UTM system.
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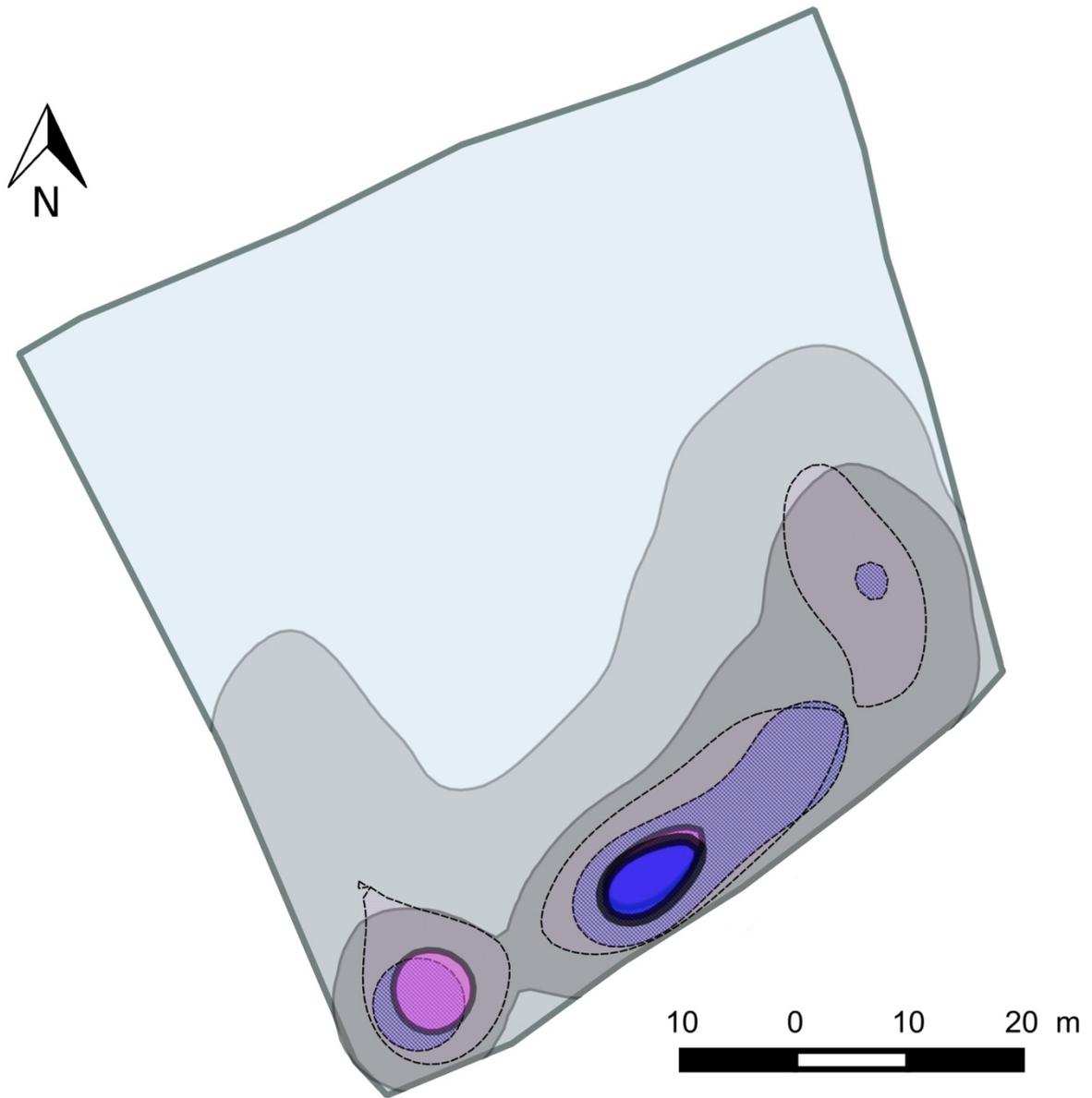
308 Figure 5. Space occupation of a mating pair of *Arapaima gigas* in the pond (3 days of
309 survey). Points represent calculated positions every 30 s. a: Male bearings (blue dots) ; b:
310 Female bearings (purple dots). c, d: grey shaded area corresponds to 95% KDE of male (c)
311 and female (d).

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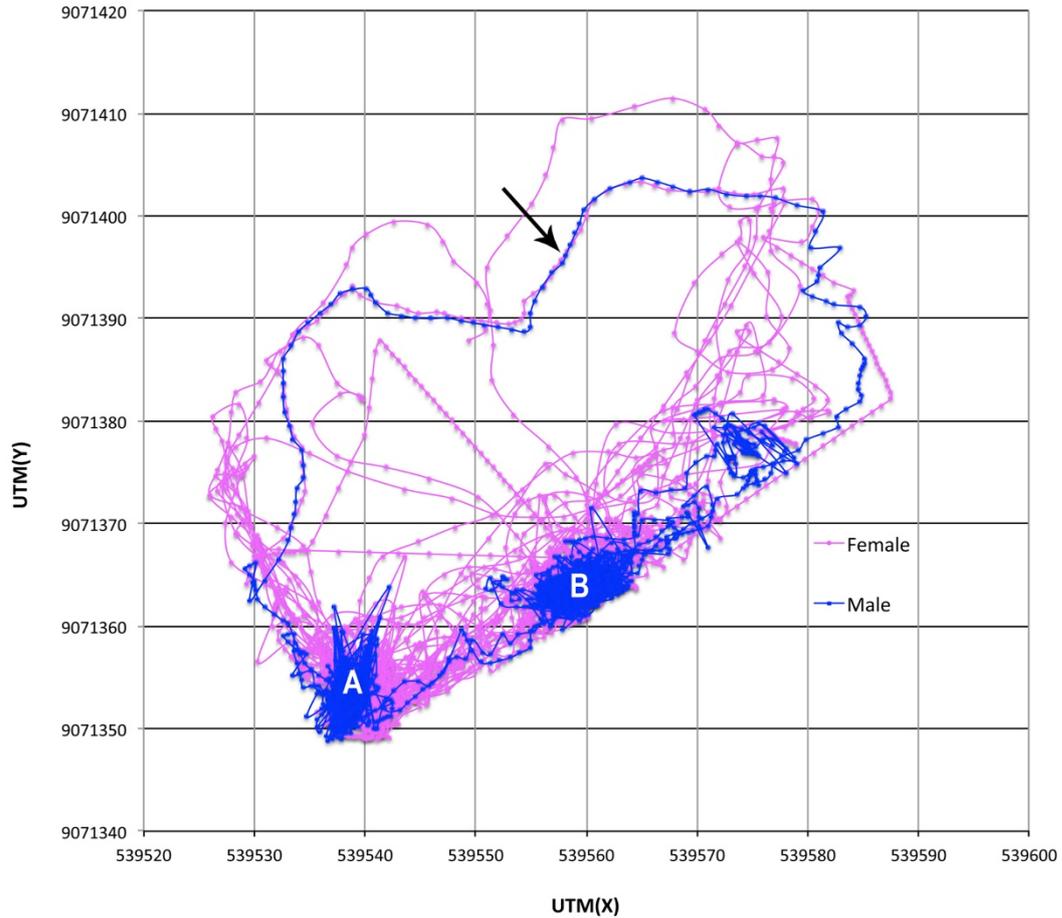
318 Figure 6. Home range areas of male and female paiches using KDE (Kernel Density
 319 estimation) at 95% (light grey : female ; dark grey: male), KDE50% (doted line shaded
 320 purple: female ; shaded blue: male) and KDE10% (solid purple: female ; solid blue: male).
 321 Each position is calculated every 30 seconds for a 2.5 days period.

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328 Figure 7. Visualization of the positions and trajectories of two paiches, Male (blue) and
 329 Female (purple) during 9.5 hours (from 0:00 to 9:30 AM) corresponding to the period of
 330 spawning. Each position is determined every 10 s. The arrow indicates a very peculiar
 331 trajectory where both male and female are travelling together towards the middle of the
 332 pond and then coming back to the nesting areas (here two areas labeled A, B).

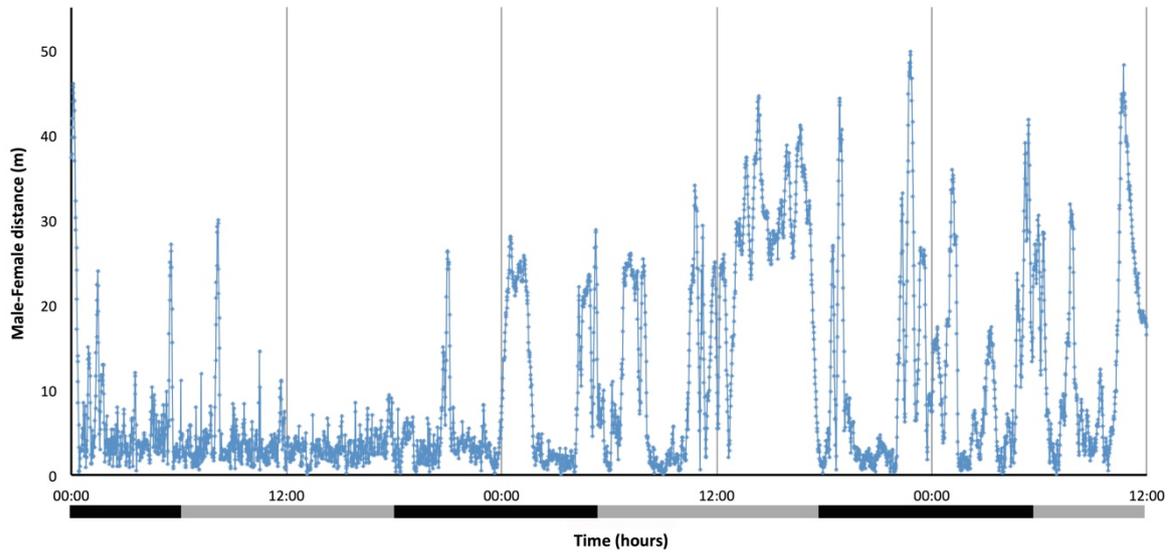
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Figure 8. Male-Female distance for a mating pair during a 2.5 days follow up in the course of a spawning event (which occurred the first day of this follow up). Data represent averaged distances of the fish calculated every minute.

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