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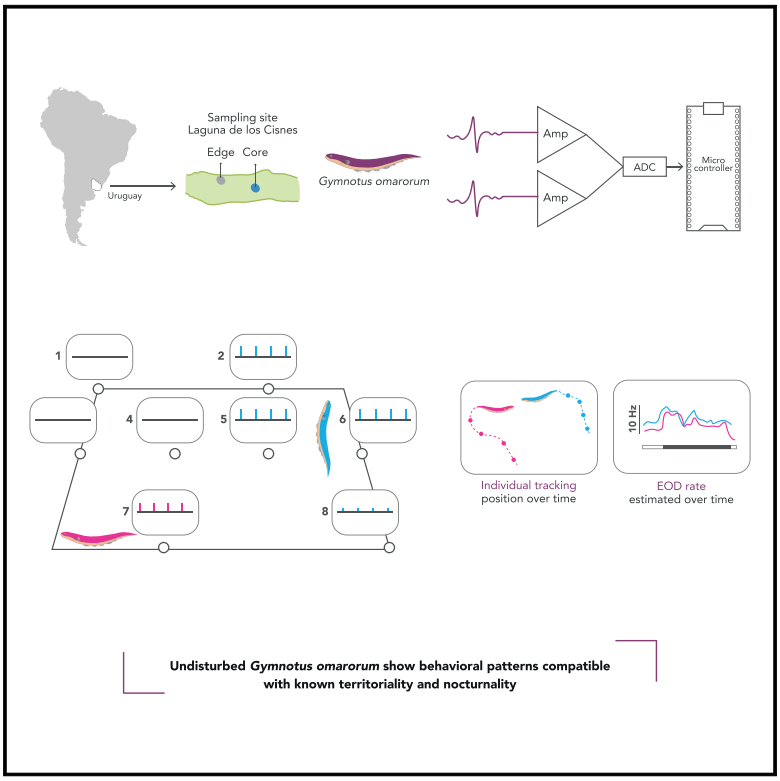
Tracking spatial patterns and daily modulation of behavior in a natural population of the pulse-type weakly electric fish, *Gymnotus omarorum*

Graphical abstract



Highlights

- Successful individual tracking of wild pulse-type weakly electric fish
- *G. omarorum* spacing patterns are compatible with known nocturnality and territoriality
- Residents keep their diurnal resting sites and move within small areas during the night
- The nocturnal electric arousal of residents is linked to daily water temperature maxima

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In brief

Behavioral neuroscience; Neuroscience

Migliaro et al., 2025, iScience *28*, 112018
March 21, 2025
https://doi.org/10.1016/j.isci.2025.112018

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Tracking spatial patterns and daily modulation of behavior in a natural population of the pulse-type weakly electric fish, *Gymnotus omarorum*

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https://doi.org/10.1016/j.isci.2025.112018

SUMMARY

Tracking individual spatial and activity-rest patterns in natural populations is challenging because it is seldom possible to monitor individual-specific traits continuously. The continuous emission of electric signals (EODs) by weakly electric fish provides a unique opportunity to do this. We present a cutting-edge technique, arrays of electrodes connected to low-cost amplifiers and tracking algorithm, to provide the individual identification of pulse-type weakly electric fish in the wild. Based only on EOD recordings of individuals of *Gymnotus omarorum*, we show that (1) there are more fish in core than in edge zones; (2) transitions into and out of the recording sites were more frequent at night, and (3) resident fish show robust nocturnal increases of EOD rate likely associated with daily variations of water temperature. This experimental approach can be extended to other species to improve our understanding of the behavior, ecology, and well-being of electric fish in natural environments.

INTRODUCTION

Tracking locomotor and activity-rest patterns in natural populations of animals remains a big challenge.[1] Most natural animal populations cannot be continuously monitored using non-invasive methods that allow to identify individuals, i.e., by recording a reliable individual-specific trait to act as a biometric cue.[2–4] However, electric fish, which continuously broadcast electric information, offer a unique opportunity to track individuals in a wide range of environments. These freshwater fish, which have evolved independently in South America and Africa, generate weak electric fields to locate and identify nearby objects (electrolocation) and to communicate with each other (electrocommunication).[5,6] Their electric organ discharges (EODs) carry information about species, sex, social status, and motivational state,[7] making them a distinct individual-specific behavioral signature.

Weakly electric fish are categorized into two groups, pulse-type and wave-type, based on the dynamics of their electric fields. Pulse-type electric fish emit brief (typically less than 2 ms), stereotyped pulses separated by longer periods of silence with a rate that lies generally below 100 Hz. In mormyriforms, the pulse rate is highly variable, whereas in gymnotiforms it tends to be more regular.[8] In contrast, wave-type electric fish generate continuous, highly periodic EODs, whose high frequencies (200–1,000 Hz) and waveforms are species- and individual-specific. Therefore, EODs can be used as a cue for individual recognition. However, several species (especially pulse-type electric fish) show dramatic plasticity in their EOD rates and waveforms, which can complicate individual identification, particularly in natural scenarios.[9,10] Recent studies have successfully tracked wave-type electric fish in the wild using their individual-specific EOD frequency.[2,11–13] However, given the context-dependent variability of EOD rate of pulse-type electric fish, individual monitoring of them has only been achieved through video tracking and machine learning, methods that are not feasible to implement in the wild.[14,15]

Gymnotus omarorum is a pulse-type weakly electric fish widely distributed in the southern boundary of gymnotiform distribution in South America.[16,17] It has been extensively studied as a model system for the understanding of the anatomical and functional principles of active electroreception,[18] as well as the neuroendocrine mechanisms underlying intra- and inter-sexual year-round territorial aggression.[19,20] Ecological data from electric censuses during the resting phase of *G. omarorum* in the wild showed a spatial distribution compatible with territoriality, i.e., with individuals evenly spaced rather than aggregated with other

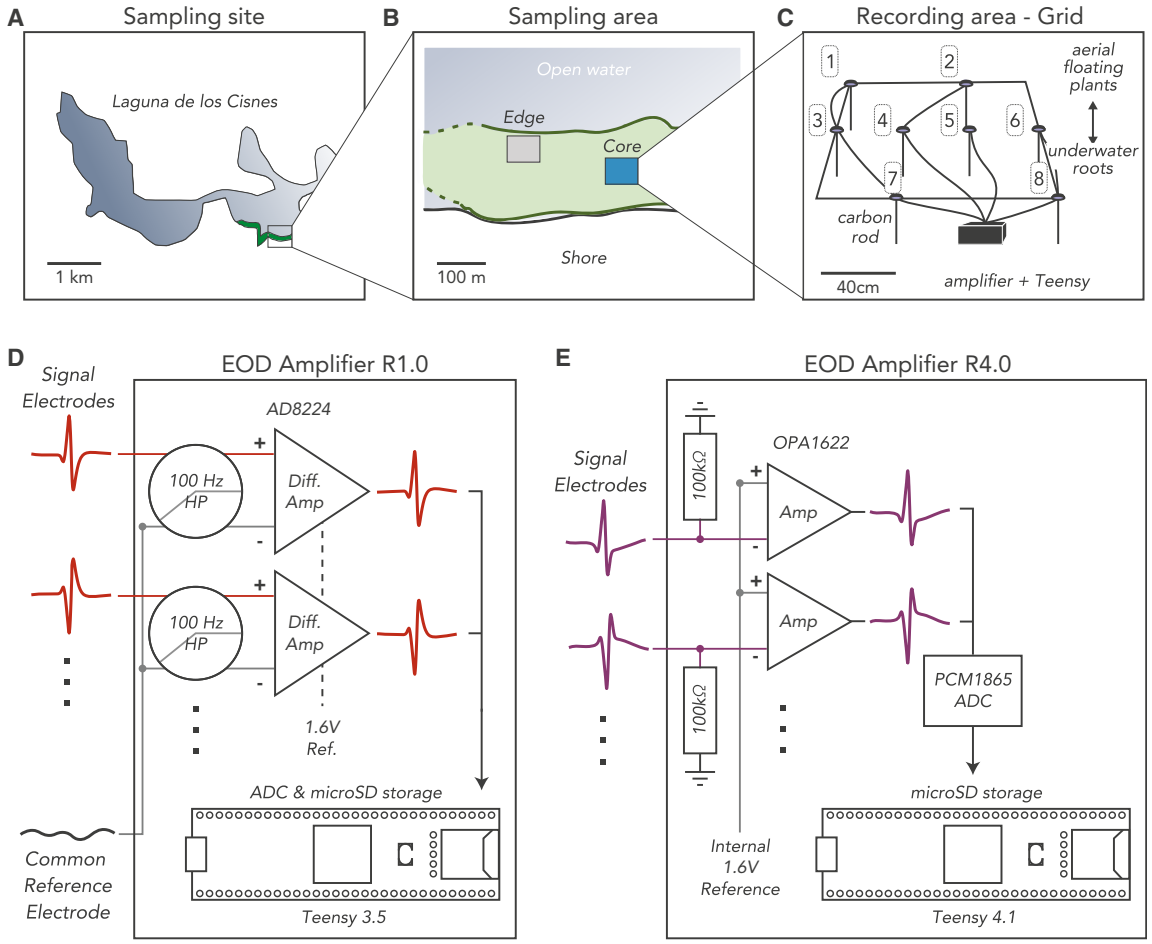

Figure 1. Representation of sampling sites and the automatized recording system

(A) The study site is located in Maldonado, Uruguay, in Laguna de los Cisnes.

(B) Water hyacinths along the shore of the lake create an extensive floating mat that defines the sampling area. The recording devices were placed close to the shore (core, blue rectangle) and closer to open water (edge, gray rectangle).

(C) Schematic representation of the recording device: an array of an eight-electrode grid system (120 × 80 cm, with electrodes spaced at 40 cm) was positioned on top of the floating mats, with the carbon electrodes (40 cm) submerged underwater through the plant roots.

(D) Schematic representation of the R1.0 EOD amplifier. Signals were high-pass filtered (HP, 100 Hz cutoff frequency) and differentially amplified with a gain of 30 against a common reference electrode that was placed at a distance of 50 cm to the recording electrodes in the water. Amplified signals were digitized with a sampling rate of 20 kHz and stored in 16-bit wave files on a microSD card using a Teensy 3.5 with onboard analog-digital converter (ADC).

(E) Schematic representation of the R4.0 EOD amplifier. Signals were coupled to battery ground (GND) via 100 kOhm resistors and amplified against an internal 1.6V reference with a gain of 10. Note that this setup does not use an external reference electrode. The signals were further amplified (gain of 10) and digitized with a sampling rate of 48 kHz using a PCM1865 ADC and stored on a microSD card using a Teensy 4.1.

conspecifics.[21] Although the typical habitat of *G. omarorum* is homogeneously covered by floating mats, individuals are more abundant in the core area, where fish rest in constant darkness, than near the edge of the plant cover adjacent to open water.[21] Previous studies have also shown that *G. omarorum* is a nocturnal fish that increases both its exploratory and electric activity during the night.[22] The nocturnal arousal of weakly electric fish can be observed by the increase in EOD basal rate that improves electroreceptive resolution and functions as an alert mechanism.[23–26] EOD recordings from identified individuals with partially restricted movements in both laboratory[26] and natural settings[22] show a robust nocturnal increase of EOD basal rate, which persists in total darkness and in motionless fish. Thus, the environmental light cycle does not appear to be a relevant trigger for the nocturnal electric arousal of *G. omarorum,* suggesting the daily water temperature cycle as an alternative synchronizing factor.[22] Efforts to answer these biologically relevant questions have faced several methodological barriers so far. Spacing and locomotor patterns of individual *G. omarorum* have yet to be assessed through long-term continuous observations, and the nocturnal electric arousal along with its temporal relation to the daily temperature cycle has never been recorded in freely moving animals in their natural habitats.

We present a cutting-edge technique that we developed specifically for this study: arrays of electrodes connected to low-cost amplifiers and algorithm for the analysis of the recorded

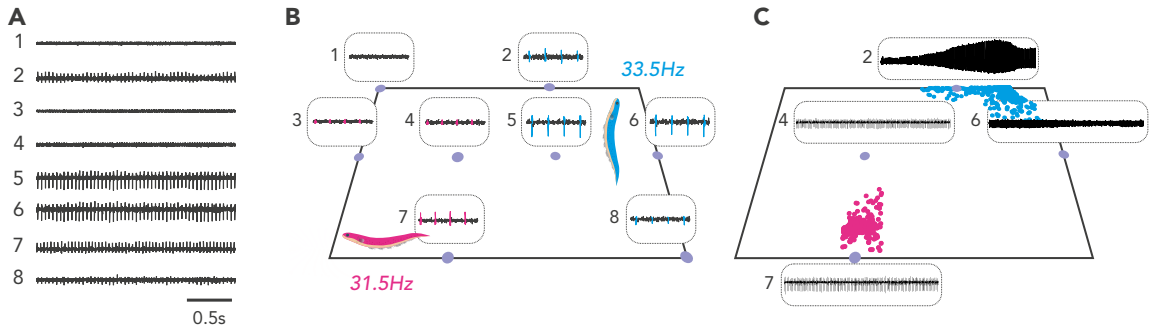

Figure 2. EOD recordings with the 8-electrode array allow for individual identification and fish position estimation

(A) Recorded traces from the 8-channel grid showing EODs for 2 s from the first 100 min recording of Core 1 sampling site.

(B) Example of fish position and identity based on EOD signals obtained from the different electrodes. In this case, two fish (magenta and cyan) were identified, and a subsequent calculation of the EOD rate was performed.

(C) Fish position over time from the example in (B). The change in EOD amplitude reflects a change in fish position. In this case, a transition between different parts of the grid can be observed for the cyan fish.

EODs. This method allows individual recognition of fish within a natural population of pulse-type weakly electric fish. The EOD recordings obtained by this non-invasive technique allowed us to infer the natural locomotor, electric, and social behaviors of *G. omarorum*. We used data from electric fields alone to address relevant open questions that previous non-automatized procedures could not resolve[21,22]: (1) Do individuals of *G. omarorum* display a differential spatial distribution across the homogeneously plant-covered littoral zone of the lake? (2) Do these individuals show day-night changes in their spatial patterns? (3) Do they exhibit the same nocturnal electric arousal as previously observed in non-freely moving animals?

RESULTS

We recorded the electric behavior of freely moving individuals of the pulse-type gymnotiform, *G. omarorum*, in their natural habitat (Laguna de los Cisnes; 34° 480 S, 55° 180 W), a 205-hectar freshwater lake in Maldonado, Uruguay, around the fall (March) and spring (October) equinox in 2023 (Figure 1). *G. omarorum* is the only gymnotiform species that occurs in this site[17] and is typically found resting during the day beneath the roots of free-floating plants that cover the littoral area of this lake (Figure 1B). Although this mat of floating plants is dense and homogeneous, a previous electrical census done in this location found that individuals of *G. omarorum* preferred to rest at the core of this homogeneous littoral area but not at the edge.[21] We thus placed one recording device in the core sampling area and a second one at the edge (Figure 1B) and obtained simultaneous continuous recordings from both sampling areas. We recorded over a 1,000-min period (approximately 17 h), covering the transition from day to night, the entire night, and the transition from night to day. Recordings were taken over three nights, each night at different positions within the core and the edge sampling areas. We used 8-channel recording systems in which the eight recording electrodes were arranged in a grid structure (120 × 80 cm, with electrodes spaced at 40 cm; Figure 1C). EODs of *G. omarorum* individuals were recorded on microSD cards using low-cost, battery-powered Teensy microcontrollers and custom-made multi-channel amplifiers[27] (Figures 1D, 1E, and S1). As the size of the grid recording array matched the average estimated size of an individual's territory,[21] we were able to assess the presence and movements of all the fish present within the territory of a single individual using exclusively electrical cues obtained by non-invasive continuous recordings.

Individual identification and fish position estimation

A two-step algorithm was used to extract the positions and identities of freely moving individuals from the EOD recordings in the wild (Figures 2 and 3). This method continuously tracks fish movements and updates their identities based on positional data and EOD rates. First, the algorithm tracked the spatial position of each fish in relation to the grid geometry using the relative EOD amplitude across grid electrodes based on weighted spatial averages as previously described[13] (Figure 2B). Second, the algorithm assigned an identity to each fish through k-means clustering of spatial position previously obtained in the first step and merged contiguous clusters that exhibited similar EOD rates (Figure 2C).

To illustrate the discrimination power of this tracking algorithm, Figure 2A shows 2 s of raw recordings from the first 100 min of recording at one of the core sampling sites (Core 1 in Figure 3), where five out of the eight electrodes of the grid system captured EODs. The tracking algorithm identified the presence and position of two distinct individuals of *G. omarorum* (magenta and cyan fish) underneath the grid system (Figure 2B) and tracked the movement of one of them (cyan fish) as it transitioned from electrode #6 to electrode #2 (Figure 2C). Once each detected fish was identified individually, we calculated their EOD rates (as the mean value from stable 1-min EOD recordings) and analyzed their variations throughout the day-night-day transitions. Figure 2B shows that the cyan fish had a slightly higher EOD rate (33.5 Hz) than the magenta fish (31.5 Hz). This approach ensures accurate identification even when fish overlap spatially or their signals change at the same time.

We never observed more than four fish in the same sampling area. In these cases, the tracking algorithm worked to

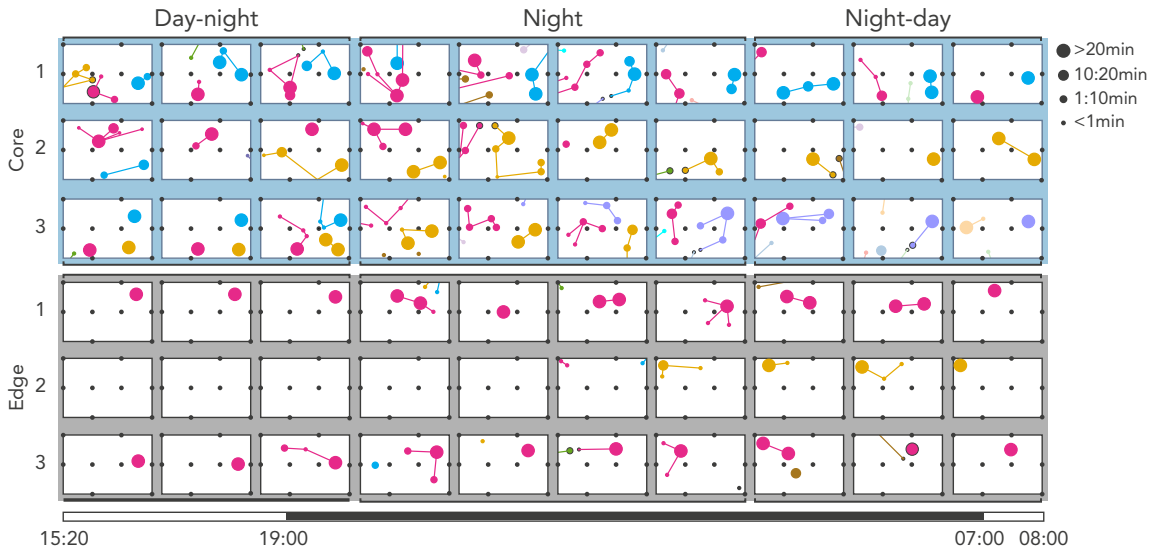

Figure 3. Distinctive spatial patterns between core and edge sampling areas

Data obtained from the monitoring of six sampling sites, with three situated closer to the shore (core, 1–3 in upper rows, light blue) and three closer to the open water (edge, 1–3 in lower rows, gray), recorded from 15:20 to 08:00 in ten 100-min windows. A total of 44 fish are represented in this figure; each tracked individual is represented by a dot, distinguished by its distinctive color. In each sampling site, the first identified fish was marked in magenta, the second in cyan, the third in yellow, and so on, enabling tracking of its position over the 1,000-min recording time. The size of the dots in each 100-min window reflects the duration for which each fish was recorded in the tracked position, whereas transitions between positions are indicated by connecting lines. Eleven fish were categorized as residents (when they were tracked for more than 60 min in the day-night and/or night-day transitions): C1R1 (magenta fish in row Core 1), C1R2 (cyan fish in row Core 1), C2R1 (magenta fish in row Core 2), C2R2 (yellow fish in row Core 2), C3R1 (magenta fish in row Core 3), C3R2 (cyan fish in row Core 3), C3R3 (yellow fish in row Core 3), C3R4 (purple fish in row Core 3), E1R1 (magenta fish in row Edge 1), E2R1 (yellow fish in row Edge 2), E3R1 (magenta fish in row Edge 3).

discriminate the individual identities of the four fish present. In the example shown in Figure S2, the EODs from four individuals of *G. omarorum* were captured by several electrodes (2, 4, 5, 6, and 7). The EOD of two of the detected fish (purple and brown dots) were only observed at one single electrode (4 and 7, respectively) and were effectively located close to their respective electrodes. The other two fish (magenta and cyan) were detected by multiple electrodes and were located in intermediate positions among electrodes 2-4-5-7 (magenta fish) and 2-5-6 (cyan fish).

More individuals were identified in the core than in the edge

We tracked 44 individuals of *G. omarorum*, 31 at the core sampling sites and 13 at the edge sampling sites (Figures 3 and 4A). Figure 3 provides a comprehensive view of the day-night spatial patterns of *G. omarorum* in the wild, including individual position and movements, the distinction between residents and visitors, day-night changes, and social interactions. This figure shows the data obtained from the six sampling sites, three located at the core of the littoral area (upper rows, light blue background) and three located at the edge of the littoral area (lower rows, gray background) from 15:20 to 08:00 in ten 100-min windows. For each of the six recording areas, each tracked individual is shown as a dot, whose distinctive color allows us to follow its position across the 1,000-min recording time. In each 100-min window, the size of the dots represents the duration each fish was detected in the tracked position. Transitions between positions are marked with a connecting line.

We identified individuals of *G. omarorum* as residents if their presence was detected for more than 60 min either during the first 300 min (day-night transition) or during the last 300 min (night-day transition). We thus identified eight residents in the core sampling areas and three residents in the edge sampling areas (Figures 3 and 4A). More visitors were detected in the core sampling areas (n = 23) compared to the edge sampling areas (n = 10; Figure 4A). Only one resident fish was recorded at each of the three edge sampling sites (Figure 3, lower rows). While up to three fish were identified as residents of core sampling sites in the day-night transition, only one resident fish remained in each core sampling site during the night-day transition (Figure 3, upper rows). The dynamic transitions of the individuals recorded at each core sampling site give further insight into the spatial patterns of individuals in Figure 3 (upper rows). In the first row, the same two individuals identified as residents at the beginning of the recording period (magenta and cyan) remained on site until the end and occupied the same position within the grid in the morning as they had before sunset. In the second row, one individual was identified as resident of this core sampling site at first (magenta), but a second fish arrived later and remained as the only resident by the end of the night. In the third row, three resident fish were identified at the beginning of the recording period (magenta, cyan, and yellow) but none remained until the end of the night.

Day-night changes in spatial patterns

The nocturnal increase of *G. omarorum* activity is evident as more fish and more individual fish movements (transitions)

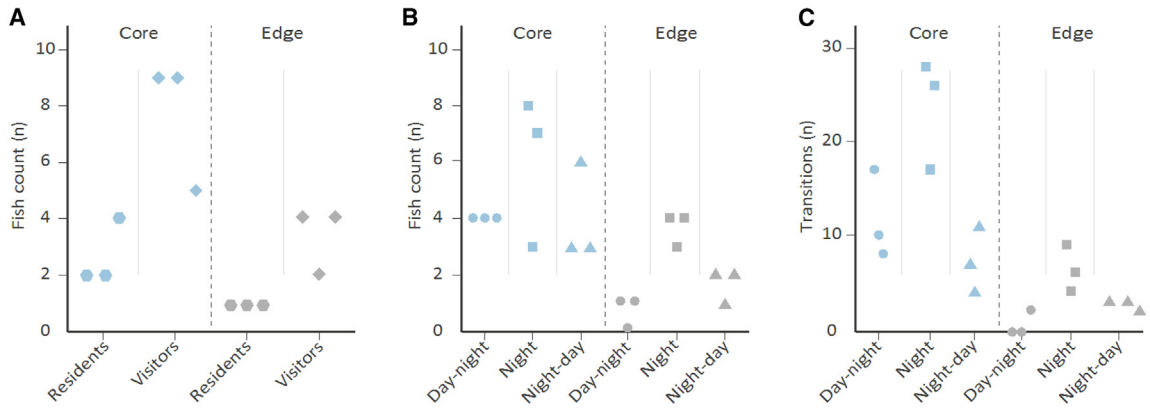

Figure 4. Day-night changes in spatial patterns

The three core areas (blue symbols) show more fish than the three edge areas (gray symbols), whereas more fish were detected during the night in both types of sampling sites. Each symbol represents the number of fish/transitions found in one sampling site (either Core or Edge) during the 1,000-min recording period.

(A) Number of residents (hexagons) and visitors (diamonds) found in the three Core and in the three Edge sampling areas.

(B) Number of fish found in the three Core and in the three Edge sampling areas during the night-day (circles), night (squares), and night-day (triangles).

(C) Number of fish movements (transitions) found within the three Core and in the three Edge sampling areas during the night-day (circles), night (squares), and night-day (triangles).

were detected during the night in each of the sampling sites (either core or edge, Figures 4B and 4C). Additionally, more fish and more individual fish movements were detected in core sampling sites than in edge sites during the night (Figures 4B and 4C). Interestingly, especially during the night-day period, the overall spatial patterns of core and edge sites became more similar, with a similar number of identified individuals and a lower number of individual position changes (Figures 4B and 4C).On several occasions, especially during the night, we observed the simultaneous presence of two individuals in close proximity to each other (detected by the same grid electrodes) within the grid system (dots circled in black in Figure 3). Whenever these spatial overlaps occurred, one fish left the grid afterward, while the remaining fish was always a resident.

The nocturnal electric arousal may be driven by changes in water temperature

Although EOD rate showed a high inter- and intra-individual variation during both day and night, there was a consistent nocturnal EOD rate increase (Figure 5). This increase began before sunset (at 19:00), whereas the EOD rate decrease preceded sunrise (at 07:00; see the solid white-black bar at the bottom of Figure 5 indicating the light-dark environmental cycle). Figure 5 shows the mean EOD rate obtained from all 44 detected individuals (color-coded as in Figure 3) across the six sampling sites (core sampling areas on the upper row and edge sampling on the lower row) throughout the day-night-day transitions from the afternoon of the first day (15:20) to the morning of the second day (08:00). The mean EOD rates of the 11 resident fish, for which we obtained long-term recordings, were interpolated and are shown as continuous lines across the recording period (Figure 5).

The nocturnal increase of EOD rate occurred while water temperature was decreasing, and the diurnal decrease of EOD rate occurred when the water temperature was at its lowest levels (see water temperature plots displayed below each EOD rate graph in Figure 5). However, almost no light reached the core sampling areas and only dim light reached the edge areas throughout most of the recording period (see water illuminance plots below each EOD rate graph in Figure 5). As shown in Figure 6, the latencies between sunset and the peak of EOD rate (62 ± 46 min; CV: 73.97%; Figure 6A) were more variable than the latencies between the peak of water temperature and the peak of EOD rate (261.4 ± 47 min; CV: 19.88%; Figure 6B). This difference in latency variability was proven significant when comparing the residuals of the latencies EOD rate peak-sunset versus EOD rate peak-water temperature peak ($n = 8$; Wilcoxon signed-ranked test; $p = 0.001$). This suggests that the timing of the nocturnal increase of EOD rate was more closely associated with the daily peak of water temperature than with the timing of the light-dark changes (sunset).

DISCUSSION

In this study, we present a set of technologies, combining an array of electrodes for non-invasive EOD recordings with computational methods for identification and position tracking of individual pulse-type weakly electric fish in their natural habitat. Based solely on EOD recordings of *G. omarorum* individuals, we provide insights on their spacing, locomotor displays, social interactions, and day-night behavioral patterns. Our findings confirm that EODs can serve as a reliable biometric cue for monitoring of individual fish, not only in species emitting quasi-sinusoidal discharges[2,11–13] but also in pulse-type weakly electric fish. The grid recording system and tracking algorithm were developed specifically for *G. omarorum*, based on previous knowledge of this species and its natural environment.[22,23] However, this approach can potentially be extended to other species, opening a promising path for gaining further knowledge on behavior, ecology, and conservation of electric fish in their complex natural environments.

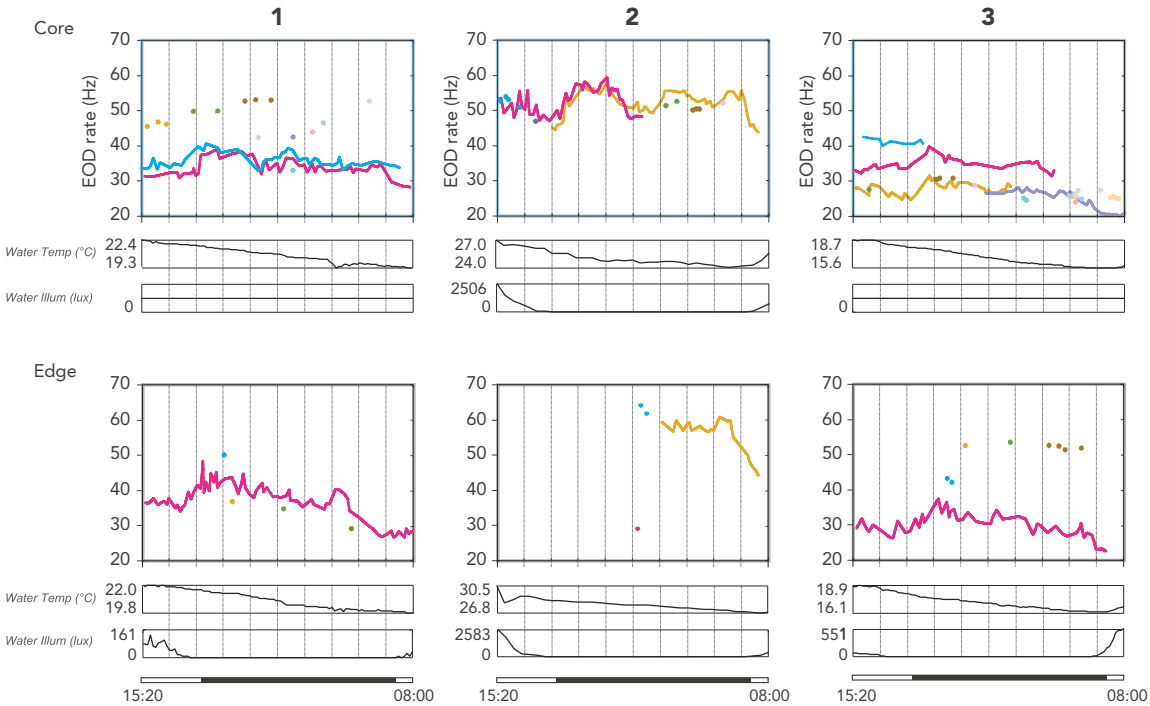

Figure 5. Individual EOD rate during the day-night-day transitions

Mean EOD rate across the whole recording period (afternoon of the first day [15:20] to the morning of the second day [08:00]). EOD rates obtained from all detected individuals (n = 44) are represented with distinctive colors (same as in Figure 3) in the six sampling sites (Core sampling above and Edge sampling below). The mean EOD rate of resident fish, based on long-lasting recordings, is interpolated and presented by continuous lines. The mean EOD rate of visitor fish is presented as dots. The solid white-black bar at the bottom indicates the duration of the external night, extending from 19:00 (sunset) to 07:00 (sunrise). Water illuminance and water temperature plots with data obtained at each Core and Edge sampling area are displayed below each EOD rate graph. Note that despite the high inter- and intra-individual variations, a consistent increase in nocturnal EOD rate is observed, preceding the onset of the external night, alongside a consistent decrease preceding the external day.

Tracking individuals of *G. omarorum* in the wild

The refinement of previously developed recording devices and tracking algorithm allowed us to track individuals of *G. omarorum* in the wild. It has long been recognized that the ability of electric fish to generate EODs is an invaluable asset for tracking their location and movements in space. Numerous studies have successfully used EODs for tracking fish in both laboratory and natural settings.[2,11–15,28–30] This study reports non-invasive tracking of a natural population of pulse-type weakly electric fish. To do this, we modified both the filter frequencies and switched from an external ground electrode to an internal common one to improve the signal-to-noise ratio of EOD recordings and therefore their resolution (Figure S1). With respect to the identification of individual position within the grid recording area, we adapted the tracking algorithm that was first developed by Henninger et al.,[13] and refined by Raab et al.[2] Although EOD rate is more flexible in pulse-type than in wave-type electric fish, it is still the main attribute for individual identification in *G. omarorum*. EOD waveform and amplitude are too variable to be reliable indicators of fish identity. We thus used EOD rate data to form clusters from the eight electrodes of the grid system in a manner similar to spike sorting in multi-unit electrophysiological recordings.[31] Each cluster was then assigned to one fish, which was facilitated by the locomotor patterns of this territorial species, whose individuals tend to move within a small area and hold the same position for hours. While two simultaneous clusters indicate the presence of two fish underneath the grid system (Figure 2B), merged clusters can also indicate positional changes of one individual within the grid area across time (Figure 2C).

Our tracking algorithm demonstrated sufficient resolution to detect up to four *G. omarorum* individuals (Figure 3). However, further refinements to our algorithm may improve its robustness against potential cases of misidentification, for example, when two fish of the same size are positioned directly on top of each other or cross each other while changing their EOD rates in synchrony. One possible improvement could be to reduce the distance between electrodes, for example, to 20 cm. A denser array of electrodes would improve triangulation and fish identification, enhancing the overall accuracy of the analysis. However, this will not completely solve the identity problem if the fish cross each other and change their EODs simultaneously.

It would also be interesting to test the discrimination power of our tracking algorithm in future experiments during the breeding season (when we expect to detect more individuals per recording area) or in species that tend to form larger aggregations.[32] The power of this approach to show seasonal behavioral dynamics within neighborhood-scale populations could be

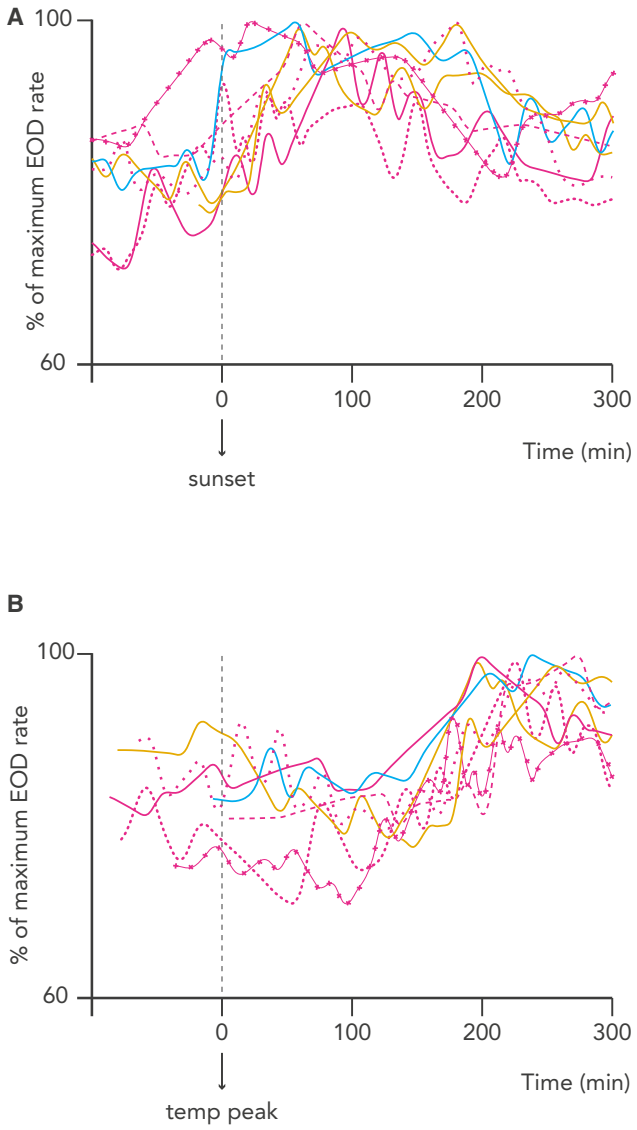

Figure 6. The nocturnal electric arousal is associated to the water temperature peak

Latency of the nocturnal increase in EOD rate in relation to sunset or the moment of water temperature peak. Each of the eight resident fish that were present on the recording sites during the nocturnal increase of EOD rate are labeled with the same color as presented in Figures 3 and 5 (magenta, cyan, and yellow for fish detected in the first, second, or third place). Given that in five of the recording sites the first detected fish is a resident, magenta lines are individualized by solid, dashed, dotted lines as well as with solid lines with symbols, for clarity.

(A) Nocturnal increase of EOD rate using sunset (19:00) as time reference. The graph shows the EOD rate as percentage of its maximum value of the eight residents from 100 min before sunset to 300 min after sunset.

(B) Nocturnal increase of EOD rate using the moment of the water temperature peak as time reference. The graph shows the EOD rate as percentage of its maximum value of the eight residents from 100 min before the water temperature peak to 300 min after the water temperature peak.

further enhanced by extending the size of the sampling area in future studies. However, increasing the area of recording will not necessarily increase the resolution of individual identification since not all electrodes in the grid will detect the EOD signal of the same fish. Simply adding more electrodes further away would have little effect in resolving this issue.

Distinctive spatial patterns between core and edge sampling areas

The continuous monitoring of *G. omarorum* individuals allowed us to confirm some previously known features of the behavior and life history of this species. First, the number of individuals found on each grid patch ranged from 3 to 13, with a maximum of three resident fish occurring simultaneously. As the size of the recording area matched the estimated territory size of a single *G. omarorum*, these findings indicate that the density of this population is higher than 1 fish per square meter, which was previously estimated based on diurnal electric censuses during the non-breeding season.[21] We were able to discriminate between residents and visitors and showed that there were more visitors than residents. However, it is important to note that, although we have a reliable categorization for residents in each recording area, some visitors may have been residents from nearby spots. Second, we detected more fish and greater movement during nighttime, as expected for a nocturnal weakly electric fish,[33] though this behavior has rarely been shown in natural unrestrained populations.[13,27,32] Third, we detected more fish (residents and visitors) and more fish movement in core sampling sites than in edge ones, which confirms that *G. omarorum* prefers to inhabit the central part of the littoral zone as previously speculated based on daytime electrical census.[21] It is remarkable that the spacing patterns of *G. omarorum* and their interactions are so different between these two microhabitats (core and edge), which are only 2–3 m apart and that do not differ in anything except the proximity of the edge to open water and, consequently, to an increased exposure to light and water movements, among other differences.

The continuous monitoring of *G. omarorum* individuals contributed to unravel some new aspects. An unexpected result was that fish, especially residents, remained within a small area during the day-night-day transitions. Likely protected and constrained by the dense roots of aquatic plants, resident fish tended to be detected within the same 1 square meter area, even during their nocturnal active phase. This unexpected stationarity, which was crucial for individual fish identification, is probably characteristic of the non-reproductive season and may not occur when reproductive drives force adult *G. omarorum* to seek mates.

Indirect evidence for the occurrence of agonistic behavior in the wild

Our recordings provide the opportunity to infer agonistic interactions of *G. omarorum* in the wild. Since early reports,[23] the genus *Gymnotus* has been recognized as highly aggressive and territorial. In particular, the non-breeding aggressive behavior of *G. omarorum* has been extensively studied in laboratory and semi-natural settings,[19,20,34] establishing it as a valuable teleost model system for the understanding of neuropeptidergic and non-gonadal steroidal modulation of aggression.[34–38] In a laboratory arena designed to mimic the natural conditions of *G. omarorum* habitat, Perrone et al.[34] showed how dyadic

agonistic encounters mediate territory acquisition and its defense. After a brief but highly aggressive contest, the dominant fish keeps the territory, monopolizes the only shelter, and excludes the subordinate from the territory every time it tries to return. Although the tracking of *G. omarorum* in the wild did not include video images, our results suggest that agonistic interactions between residents and between residents and visitors mediate territoriality in natural conditions, where losers can freely flee out and search for less contested spots. We observed transient visitors (that stayed for less than 1 min in the recording area) and visitors that were recorded within the recording area for several minutes. Although we cannot determine whether these visitors were the same fish returning or different fish, we can undoubtedly show that territories are challenged regardless of the availability of alternative spots. For example, in the second row of the core sampling sites in Figure 3, it appears that the yellow resident entered the grid area during the night, interacted with the magenta fish, and excluded it, holding the territory until the end of the recording period. It is also evident that resident fish excluded intruders (visitors) in almost all (5 out of 6) of the recording sites. Even more suggestive of agonistic interactions is the observation that in all cases (8 out of 8) where two fish interacted in close vicinity (dots circled in black in Figure 3), one of them retreated from the grid area while the other remained in the territory as a resident.

This study provides evidence of the strict site fidelity of *G. omarorum*. Site fidelity, the tendency to return to a previously occupied location, has been largely linked to territorial behavior in a wide variety of species.[39–42] Until now, individual identification of pulse-type weakly electric fish in the wild had required invasive techniques, and site fidelity was assumed but never confirmed in any territorial species of weakly electric fish. For example, serial electric censuses during consecutive days at the same location used in this study have indicated the presence of *G. omarorum* individuals in the same spot (L. Zubizarreta, personal communication). However, this procedure cannot ensure that each time a fish is detected, it is effectively the same animal. In a non-territorial pulse-type species in the wild, mark-recapture procedures showed that males and females moved around 4 m and 8 m, respectively, between successive days.[32] The results presented in the first row of the core sampling sites in Figure 3 provide robust evidence of the strict site fidelity of *G. omarorum*. The magenta and cyan fishes, which moved around the grid sample area during the night, returned to the same spot of the grid to rest before and after the nocturnal active phase. In fact, all the resident fish that remained within the grid sampling area during the whole recording period showed this precise site fidelity selecting resting sites on the following morning that were not more than 30 cm from the location where they rested the previous afternoon.

The daily cycle of electric behavior

We present evidence of the nocturnal electric arousal of natural individuals of *G. omarorum*. Day-night changes in electric behavior have been well documented in several species of weakly electric fish in laboratory settings at constant temperatures.[23–26,43] As a common trait across species, these day-night changes include a nocturnal increase in EOD rate, which has been interpreted as a nocturnal electric arousal that improves electrolocation and electrocommunication resolution during the active phase. This nocturnal electric arousal persists in motionless fish and under constant darkness in the wild while water temperature is actually decreasing.[22,43,44] A previous study carried out at the same core sampling site we used in this study showed that restrained individuals of *G. omarorum* exhibit a robust nocturnal EOD rate increase.[22] This nocturnal electric arousal occurred without changes in water illuminance (i.e., in constant darkness) but was likely synchronized by daily variations of both water temperature and social cues.[22] In this study, we obtained EOD rate data from 44 freely moving individuals of *G. omarorum.* Visitors (individual dots in Figure 5) tended to have higher EOD rates than residents, probably because they were more actively exploring around the grid sampling area than residents. The continuous recordings of several resident fish allowed us to observe in a natural population, a clear EOD rate increase during the night across sampling sites, whose amplitude was similar to that previously reported in restrained individuals of this species.[22,26] Both the increase and decrease of EOD rate preceded sunset and sunrise, respectively. Although we captured the peak of the nocturnal increase of EOD rate during the night, we cannot be certain about the timing of its minimum because the EOD rate was at its lowest values at the end of the recording period.

Temperature is a major environmental factor, that is particularly relevant for ectotherms, because of its direct effects on their metabolic processes. For example, when *G. omarorum* is subjected to short-term gradual temperature changes, EOD rate increases with temperature.[45] However, the nocturnal increase in EOD rate occurs when water temperature is decreasing after reaching its maximum (Figures 5 and 6). This opposite response to changes in water temperature suggests that temperature is acting in at least two different ways: as the well-known factor in metabolism and as a synchronizing signal from the environment. In line with previous reports,[22] we show that the onset of the nocturnal increase of EOD rate was loosely associated with the beginning of the night but tended to be more closely synchronized with the peak of the daily water temperature cycle. Temperature, as an environmental timing cue, has been less explored than light, despite being particularly relevant in the context of climate change. Among many other implications, global warming is leading to an attenuation of temperature cycles and an increased uncoupling of the photoperiod from the thermoperiod.[46]

Concluding remarks

This study contributes to the development of tools that are required for distant individual monitoring of electric fish based on their electric discharges. There is immense potential for these techniques, as collecting continuous data from freely moving individuals will enhance our understanding of biological processes such as social dynamics, ecosystem dynamics, circadian rhythms and of course will be useful for conservation. Although the role of EODs as valuable individual cues measurable by non-invasive methods has been early recognized, individual tracking in wave-type electric fish species has only been reported recently.[2,11–13] Our study provides a successful example of individual tracking of pulse-type gymnotiforms in the wild. The

individual day-night spatial and electric patterns of *G. omarorum* reveal relevant information about spacing and behavior that open interesting avenues for future research. For example, expanding the duration of continuous grid recordings will be useful to assess the circadian rhythms of locomotor and electric activity; increasing the number of grid recordings across the year will provide interesting data on seasonal changes of spacing, territoriality, and social behavior; and increasing the size of the grid recording system can reveal behavioral dynamics within neighborhood-scale populations as suggested by early studies in other species of *Gymnotus*.[47] Finally, this study showcases an approach that can be applied not only to other electric fish species but also might inspire the search of other species-specific biometric cues (e.g., vocalizations[48]) that could allow for individual identification and monitoring of a great number of species in real-life scenarios.

Limitations of the study

We recognize several limitations of this study, most of them originated from the development of new methodologies in natural settings. These limitations primarily concern the spatial and temporal coverage of electric recordings and individual tracking. First, using only eight electrodes located 40 cm apart limited the spatial coverage of the grid and the acuity of the fish position estimation. Second, the identification of daily spatial patterns would have benefitted from longer recordings, whereas sampling the population across the year will give insight on seasonal modulations. Third, tracking algorithm succeeded in identifying up to four *G. omarorum* individuals, but their discrimination power should be tested in more crowded environments. Fourth, grid recordings and tracking analysis were developed specifically for *G. omarorum* and need to be tested and adapted to other electric fish species. On the other hand, several operational decisions made during data processing can be considered as arbitrary, such as the categorization of residents or the time delay of 10 min for a fish that leaves the grid sampling area to be considered as a different fish if an EOD of similar rate was detected. Finally, body size, which is the major determinant of the agonistic outcome in this species, could not be estimated by the tracking algorithm because the position of the animal has more influence on the amplitude of EOD recordings than body size.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources, data, and codes should be directed to and will be fulfilled by the lead contact, Ana Silva (asilva@fcien.edu.uy).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- EOD-R datasets generated and analyzed during the current study are available in the Mendeley Data: https://data.mendeley.com/datasets/hp8r5r8c26/1 and are publicly available as of the date of publication and listed in the key resources table.
- Original codes available at GitHub (Arduino C++ code for the data loggers) and listed in the key resources table: https://github.com/janscience/TeeRec, https://github.com/janscience/TeeGrid/tree/main/8channel-logger, https://github.com/janscience/TeeGrid/tree/main/R40-logger, https://github.com/fpedraja/Grid_fish_tracking.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact, Ana Silva (asilva@fcien.edu.uy), upon request.

ACKNOWLEDGMENTS

This research was partially funded by CSIC, Universidad de la República, Uruguay, Programa de Grupos I+D – 2018 # 92 to A.S. and by DFG grant 501755140 to J.B.

AUTHOR CONTRIBUTIONS

Conceptualization, A.S., J.B., A.M., and F.P.; methodology, J.B., A.M., F.P., and S.M.; software, J.B. and F.P.; formal analysis, F.P. and A.M.; investigation, A.S., J.B., A.M., and F.P.; resources, A.S. and A.M.; writing—original draft, A.S. and A.M.; writing—review & editing, A.S., J.B., A.M., F.P., and S.M.; supervision, A.S. and J.B.; funding acquisition, J.B. and A.S.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2025.112018.

Received: June 26, 2024
Revised: September 21, 2024
Accepted: February 10, 2025
Published: February 13, 2025

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
EOD rate values	Mendeley data	https://data.mendeley.com/datasets/hp8r5r8c26/1
Experimental models: Organisms/strains		
Gymnotus omarorum	nature	N/A
Software and algorithms		
TeeRec	Github	https://github.com/janscience/TeeRec
8-channel logger	Github	https://github.com/janscience/TeeGrid/tree/main/8channel-logger
R4.0 logger	Github	https://github.com/janscience/TeeGrid/tree/main/R40-logger
Tracking and identification	Github	https://github.com/fpedraja/Grid_fish_tracking
Other		
Teensy 3.5 & 4.1 Microcontroller	PJRC	https://www.pjrc.com/teensy/
TeensyAmp R1.0	Github	https://github.com/muchaste/Teensy_Amp/tree/main/R1.0
TeensyAmp R4.0	Github	https://github.com/janscience/Teensy_Amp/tree/main/R4.0

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Fieldwork was performed in Laguna de los Cisnes, Uruguay (205 ha, 34° 480 S, 55° 180 W), which consists of a three-part interconnected shallow system of freshwater (maximum depth 5 m) with no inputs of salt or brackish water (Figure 1A,[21]). The experiments were conducted during the non-breeding season at the peri-equinox period (March 10-12, 2023 and October 10-12, 2023) under a natural light-dark cycle of 12:12. Periodic light and temperature measures were taken each 30 min, inside the water close to the recording sampling sites (HOBO-MicroDAQ: UA-002-08). Measurements range: temperature, −20° to 70°C (−4° to 158°F); light, 0 to 320,000 lux (0 to 30,000 lumens/ft2).

The study species, *Gymnotus omarorum*,[16] is the sole species of weakly electric fish present in this study area.[17] The littoral area of the lake is blanketed by a dense strip (5–40 m width) of free-floating aquatic macrophytes that cover the sampling area (Figure 1B), with *G. omarorum* typically resting among the roots of these plants. Data was collected from a freely moving natural population. As illustrated in Figure 1B, two grid systems were placed on top of the natural floating vegetation, one closer to the shore (aka Core, light blue) and another in the outermost limit of the natural vegetation patch (aka Edge, gray). Each grid system consisted of an array of eight electrodes distributed on a $1m^2$ surface separated by 40 cm between each other. Based on a series of test recordings, we selected this electrode arrangement as the one that maximized the spatial resolution of fish position estimates (Figure 1C), while covering a reasonably sized area given eight recording electrodes. Consequently, this grid array assured the detection of the EODs of any individual of *G. omarorum* in any position within the recording area. The area of approximately $1m^2$ covered by the 8-electrodes array roughly matches the size of a single *G. omarorum* territory, according to electric census estimates.[21] We thus used this single-territory size as sampling area to assess the presence and movements of all the fish that were actually present there during prolonged continuous recordings.

Due to the nocturnal habitats of *G. omarorum,* recordings (from 15:20 to 08:00) focused on the night and the day-to-night and night-to-day transitions during three nights. As shown in Figure 3, this recording period was divided in 3 epochs: 1) the day-night transition (first 300 minutes); 2) the night involving 400 minutes of recording; and 3) the night-day transition (last 300 minutes).

General protocols of *G. omarorum* handling in the wild were approved by the institutional Ethical Committee for the use of experimental animals (Facultad de Ciencias, Universidad de la República, CEUA-1304). Given that this study did not actually involve animal collection nor manipulation, we cannot report the influence of sex on our results. However, previous reports obtained from the same recording site as in this study indicate that *G. omarorum* has a 1:1 sex ratio.[21]

METHOD DETAILS

Electrical recordings were made using eight carbon rod electrodes (50 cm long) which were placed below the plant coverage that reaches 30 cm under the water surface. In approximately the same water depth, *G. omarorum* is usually found resting among the roots.[22] Data were recorded over three nights (3/10/2023, 10/10/2023, 10/11/2023). Two recording systems were used. All edge

recordings and the 3/10/2023 core recordings were made using the Teensy_Amp R1.0 (https://github.com/janscience/Teensy_Amp/tree/main/R1.0) with 30× differential amplification against a reference electrode in 50 cm distance, 100 Hz high-pass filter, 7 kHz low-pass, digitized with 12 bits at 20 kHz by a Teensy 3.5 microcontroller (www.pjrc.com), and stored in continuous 1 minute wave files on μSD cards for offline processing. The 10/10/2023 and 10/11/2023 core recordings used the newer 8-channel Teensy_Amp R4.0 (https://github.com/janscience/Teensy_Amp/tree/main/R4.0, jlm Innovation, Tübingen, Germany) with 10x inverting preamplifiers and a separate analog-digital-converter (two PCM1865, Texas Instruments) that digitized the signals with 16bit at 48kHz controlled by a Teensy 4.1 microcontroller (www.pjrc.com) with additional 8MB PSRAM chip for buffering the data. The Teensy_Amp R4.0 input signals are connected via 100kΩ to common ground and thus do not require a separate reference. Logger software for both systems is based on the TeeRec library (https://github.com/janscience/TeeRec) and the "8-channels-logger" and "R40-logger" sketches of the TeeGrid project (https://github.com/janscience/TeeGrid). The custom-built recording systems were powered by phone power bank batteries (5 V, 10-20 Ah). Recordings were analyzed with Matlab routines for EOD detection and EOD rate calculation.

The key differences between the two recording systems are whether an external reference electrode is needed (R1.0) and whether the onboard ADC of the microcontroller (R1.0) or a separate ADC chip (R4.0) is used. In Figure S1 recordings of the two systems are compared. In the lake, 50 Hz noise is quite strong due to human influences (e.g., power lines), and harmonics of 50 Hz extend over the whole spectrum. Finding the right position of the reference electrode of the R1.0 system is crucial. In the wrong position the 50 Hz noise becomes too dominant, making a proper detection of EODs impossible. Further, if electric fish are located nearby the reference electrode, their signals appear on all 8 channels of the recording, acting as confounding factors for estimating the number and position of fish. The R4.0 system avoids the need of a reference electrode by tying the floating signals via 100 kOhm resistances to the internal battery ground. This ground effectively follows the average signal over the 8 channels and this way removes most of the external noise. However, this averaged ground implies that an electrode without a nearby EOD shows a negative image of an EOD recorded on another electrode. For our study, however, this did not pose a problem, because of the high noise floor that buried these negative images. The R1.0 system has an input range of 0 – 3.3V. The ADC of the Teensy microcontroller used by the R1.0 system effectively uses 12bit and is influenced by voltage changes induced by writes to the SD card. The R4.0 system also has an input range of 0 – 3.3V. The separate ADC used by the R4.0 system internally uses 24bit, does not suffer from SD-write artifact and has a much lower noise floor under ideal conditions. In addition, a single PCB of R1.0 amplifies 2 channels. We used 4 of the R1.0 PCBs to assemble an 8-channel amplifier. A single PCB of the R4.0 system amplifies and digitises 8 channels. The new R4.0 system thus provides a compact and easy to use solution for multi-channel recordings of EODs. A recent modification of the R4.0 provides 16 channels (https://github.com/janscience/Teensy_Amp/tree/main/R4.1-R4.2).

Algorithm for the estimation of fish position

The presence of individuals *G. omarorum* relied on detecting EODs in one or more of the electrodes distributed in the grid array (Figures 2A and S2; https://github.com/fpedraja/Grid_fish_tracking). The voltage traces in each electrode were bandpass-filtered (Butterworth filter, third order, 300-2000Hz). From this filtered data the peak-to-peak amplitude A_i of the detected brief EOD pulse was obtained for all eight electrodes. EOD amplitude was then used to estimate the location of each fish following Henninger et al.[13] as shown in Figure 2B. The fish position was estimated from the amplitudes A_i at the n=8 electrodes i at position e_i as a weighted spatial average, given by:

$$\vec{x} = \frac{\sum_{i=1}^{n} \sqrt{A_i} \cdot \vec{e}_i}{\sum_{i=1}^{n=z} \sqrt{A_i}}$$

The EOD of a fish can cause a very large amplitude on nearby electrodes because of the electric field's reciprocal dependence on distance. This effect results in a relatively large localization error, if a simple weighted spatial average is used, because the position estimate is pulled towards the strongest electrode. The localization error is reduced by using the square root of the EOD amplitude, √Ai, as a weight, which reduces the impact of electrodes with large EOD amplitudes (Henninger et al., 2020).

Algorithm for individual identification

Individual fish were identified based on a cluster analysis and merged over time according to their EOD rate and position between analyzed files (Figure 2C). The primary assumption for EOD detection was that the EODs of pulse-type weakly electric fish are brief signals with almost no temporal coincidence. Using the x and y coordinates of the positions obtained as described in the previous section, DBSCAN was applied to group the data points into clusters. DBSCAN was selected because it does not require a predefined number of clusters, is robust to noise, and does not assume a spherical distribution of points—allowing for more flexible and realistic cluster shapes. The EOD rate for each cluster was then calculated based on the assigned points.

As with almost any classification algorithm, some errors can be expected. In our case, fish identity is first based on fish position and orientation, and then on fish EOD rate. For an error to occur when two fish share the space, they would need to be of the same size and lie directly on top of each other. Movement of the fish within the region is later matched by their EOD rate. If two fish move and cross each other and simultaneously change their EOD rates, this can result in an error in their identification. To minimize errors, our system continuously tracks fish movements and updates their identities based on a combination of positional data and EOD frequencies. This approach helps ensure that even when fish overlap or their signals change, accurate identification is maintained

as much as possible. Although this tracking system worked nicely to discriminate up to four individuals sharing the grid area simultaneously (Figure S2), further refinements to our algorithm may improve robustness against these rare cases of misidentification. If this fish moves, the EOD amplitude detected by each electrode will change and eventually a change in the position of the fish will be detected, this in turn will generate a new cluster. To account for this drift in signal a post-analysis was performed to merge contiguous clusters with similar EOD rates (<10% difference, connecting lines between dots in Figure 3). Clusters in between files were merged using both spatial and EOD rate criteria. Only clusters with at least 100 EODs will be recognized as one individual of *G. omarorum* located at a certain position within the grid (dots in Figure 2). When the identified fish leaves the grid recording area, EODs will no longer be detected and the fish will be considered gone, unless a fish with the same EOD rate is detected again in less than 10 minutes. Given the previously reported inter-individual distance and the speculated high site fidelity of *G. omarorum*,[21] this approach proves to be the most feasible for individual identification.

Fish classification

Individuals *G. omarorum* detected within the grid system were categorized as residents or visitors. We recognized as residents those fish whose presence was identified for more than 60 min either during the first 300 min (day-night transition) or the last 300 min (night-day transition). We recognized as visitors those fish whose presence was identified for shorter periods throughout.

QUANTIFICATION AND STATISTICAL ANALYSIS

EOD rate was calculated as the inverse of the inter EOD interval. A mean EOD rate was calculated for each 1 minute to be shown in Figure 5 across the recording period for all tracked animals. The mean EOD rates of the 11 resident fish, for which we obtained long-term recordings, were interpolated and are shown as continuous lines in Figure 5. Due to the interindividual differences in EOD basal rate, changes in rate were normalized to allow comparisons among individuals as shown in Figure 6.

To evaluate the association of the nocturnal increase of EOD rate with environmental variables, we calculated the latency in minutes between the sunset and the peak of EOD rate and the latency in minutes between the peak of water temperature and the peak of EOD rate.

Time associations between light and temperature cues with the nocturnal increase in EOD rate were compared for the n=8 fish present in one of the recording areas at the moment of the nocturnal increase as shown in Figure 6. We used the nonparametric Wilcoxon signed ranked test (PAST[49]) to compare the latencies residuals between the moment of the individual maximum nocturnal EOD rate and the moment of sunset and water temperature maximum, respectively, in each recording site. We presented the values of the latencies in median ± MAD. Values of $p < 0.05$ were considered statistically significant.