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Daily rhythm of locomotor and reproductive activity in the annual fish *Garcialebias reicherti* (Cyprinodontiformes: Rivulidae)

Correspondence:
Adriana Migliaro
amigliaro@fcien.edu.uy

Andres Olivera[1], Carlos Passos[1], Juan I. Vazquez[1,2], Bettina Tassino[1,3] and Adriana Migliaro[1,2,4]

Daily rhythms of behavior and their synchronization in relevant social contexts are fundamental for the survival and reproductive success of all animal species. South American annual fish are adapted to extreme environmental conditions, where the ponds they inhabit dry out as the year progresses, while engaging in reproductive behavior from sexual maturity to death. The ever-changing environmental cycles these species are subjected to makes them an excellent model for studying the expression of biological rhythms in nature. In this work we show for the first time that *Garcialebias reicherti*, an annual fish native to Uruguay shows daily rhythms in both their locomotor and reproductive behavior. This species shows diurnal behavioral patterns, with neither sex nor reproductive context affecting the phase relationship between the light/dark cycle and activity. However, reproductive context modulates the amount of locomotor activity and leads to synchronization between members of the dyads, while introducing a second behavioral rhythm for reproductive events. Reproductive context emerges as a significant modulator of rhythmic behavior, driving circadian rhythms synchronization alongside environmental zeitgebers, while illuminating the complexity of physiological and behavioral coordination.

Keywords: Behavioral timing, Circadian rhythms, Reproductive behavior, Social synchronization.

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1 Grupo Cronobiología, CSIC, Facultad de Ciencias, Universidad de la República Uruguay. Iguá, 4225, 11400 Montevideo, Uruguay. (AO) aolivera@fcien.edu.uy, (CP) 57naranja@gmail.com.

2 Instituto de Investigaciones Biológicas Clemente Estable, Departamento de Neurofisiología Celular y Molecular, IIBCE-MEC. Av. Italia 3318, 11600 Montevideo, Uruguay. (JIV) jvazquez1066@gmail.com.

3 Sección Etología, Facultad de Ciencias, Universidad de la República Uruguay. Iguá, 4225, 11400 Montevideo, Uruguay. (BT) tassino@fcien.edu.uy.

4 Laboratorio de Neurociencias, Facultad de Ciencias, Universidad de la República Uruguay. Iguá, 4225, 11400 Montevideo, Uruguay. (AM) amigliaro@fcien.edu.uy (corresponding author).

Los ritmos diarios de la conducta y su sincronización en contextos sociales relevantes son fundamentales para la supervivencia y el éxito reproductivo. Los peces anuales sudamericanos están adaptados a condiciones ambientales extremas, donde los charcos que habitan se secan a medida que avanza el año, mientras despliegan conducta reproductiva desde la madurez sexual hasta su muerte. Los ciclos ambientales extremos a los que estas especies están expuestas las convierten en un modelo excelente para estudiar la expresión de los ritmos biológicos en la naturaleza. En este trabajo mostramos por primera vez que *Garcialebias reicherti*, un pez anual nativo de Uruguay, muestra ritmos diarios tanto en su comportamiento locomotor como reproductivo. Esta especie muestra patrones de comportamiento diurnos, sin que el sexo ni el contexto reproductivo afecten el enganche de fase entre el fotoperiodo y la conducta. Sin embargo, el contexto reproductivo modula la cantidad de actividad locomotora y promueve la sincronización entre los integrantes de las díadas, al tiempo que introduce el comportamiento reproductivo como otro ritmo conductual. El contexto social emerge como un modulador significativo de la conducta rítmica, funcionando en conjunto con los *zitegebers* ambientales, poniendo en evidencia una compleja coordinación fisiológica y conductual.

Palabras clave: Comportamiento reproductivo, Conducta reproductiva, Ritmos circadianos, Sincronización social.

INTRODUCTION

Living organisms exhibit physiological and behavioral daily rhythms stemming from the oscillations of endogenous circadian clocks. These clocks are in turn synchronized with environmental cycles or zeitgebers, among which the light/dark cycle is the most widespread (Aschoff, 1978; Aschoff *et al*., 1982; Foster, Helfrich-Forster, 2001; Ashton *et al*., 2022). Synchronization allows for better energy allocation, prediction of changes in the environment and coordination of social activities. In this sense fitness is potentiated by biological rhythms (Kumar, 1997; Paranjpe, Sharma, 2005; Dardente, Cermakian, 2007; Nikhil, Sharma, 2017) through the coordination of metabolic processes (an intrinsic adaptive value) and by synchronizing physiology and behavior with environmental cycles (an extrinsic adaptive value) (Sharma, 2003).

Locomotor activity is a standardized reference for assessing circadian rhythmicity throughout a wide variety of species, as a single output reflecting the coordination of physiological and behavioral states (Hurd *et al*., 1998; Beale *et al*., 2013; Cascallares *et al*., 2018; Starnes, Jones, 2023). In this sense animals are classified as nocturnal, diurnal or crepuscular according to the moment of the daily cycle (*i.e*., the phase of the light/dark cycle) in which the species concentrates the greatest percentage of daily movement (Herrero *et al*., 2003; Schulz, Leuchtenberger, 2006). However, this classification might be hijacked by individual variation within a single species (Helfman, 1986; Phillips *et al*., 2019) by variations associated to seasonal cycles (Tomotani *et al*., 2012), or because of ontogeny (Roennemberg *et al*., 2007; Krylov *et al*., 2021). Moreover, unpredictable environmental events can produce changes in established cycles (Prokkola, Nikinnma, 2018; Amichai, Kronfeld-Schor, 2019). Social interactions also influence daily activity patterns, highlighting the importance of contextual factors in behavioral analysis (Davidson, Menaker, 2003; Favreau *et al*., 2009; Migliaro *et al*., 2018; Gascue *et al*., 2020). Since survival depends on the successful occupation of spatial and temporal niches the presence of conspecifics might modulate their value (Larson *et al*., 2004; Eban-Rothschild, Bloch, 2012; Fuchikawa *et al*., 2016; Mildner, Roces, 2017). Moreover, group formation in teleosts modifies the pattern of nocturnal or diurnal habits (Kavaliers, 1980). Physiological and behavioral synchronization is crucial during the reproductive season influencing reproductive success (Rad *et al*., 2006). Social synchronization might change as animals undergo hormonal changes that might affect the circadian system, through the modulation of sensitivity to social stimuli (Lumineau *et al*., 1998; Campos-Mendoza *et al*., 2004).

Annual fish have the shortest life cycle of all vertebrates, one that is synchronized with the wax and wane of the freshwater ponds in which they live. Eggs hatch when ponds are full of clear water (mid-autumn, April-May) and as life progresses water turbidity increases as water level decreases until complete dry-out (summer, December-January) (Passos *et al*., 2021). As a result, natural photoperiod changes with the seasons, not only in length and phase, but also in amplitude. This raises interesting questions on the role of this typical zeitgeber on the synchronization of natural behavior. Pioneer studies on daily activity patterns of annual fish have been carried out in African species of the genus *Nothobranchius* Peters, 1868, reporting peaks of locomotor and reproductive activity close to midday (Haas, 1976; Lucas-Sanchez *et al*., 2011, 2013, 2015; ŽáK *et al*., 2019). Until now, no studies have been published on daily rhythms in South American annual fish, even though animals living under extreme environmental cycles make great models to study the synchronization of biological rhythms (Kronfeld-Schor *et al*., 2013; Migliaro *et al*., 2018; Castillo *et al*., 2023). *Garcialebias reicherti* previously known as *Austrolebias reicherti* (Loureiro & García, 2004) by Alonso *et al*., 2023 is endemic to the seasonal wetlands of eastern Uruguay (*ca*. 32°55'S 53°54'W). Adult fish engage in continuous reproduction from sexual maturity to death. They exhibit both behavioral and morphological dimorphism characterized by visual signals and locomotor displays that highlight the importance of visual information for the species (García *et al*., 2008; Passos *et al*., 2015). Males are larger than females with dark vertical bands on their body flanks, with unpaired fins and a strongly pigmented opercular region. Females are cryptic, light brown and not aggressive (Passos *et al*., 2015). Fertilization in this species is external and the courtship entails the display of locomotor patterns of attraction and response in both males and females, ending with the partial or total burial of the pair and the laying of eggs in the substrate (García *et al*., 2008; Passos *et al*., 2015). As the natural pond dry-out becomes imminent and stress levels increase, reproduction remains a highly motivated activity, despite the deterioration of environmental conditions (Passos *et al*., 2021). The importance of this social behavior demands precise synchronization in order to maximize the probability of occurrence of reproductive events. Moreover, since energy expenditure needs to be tightly controlled it is tempting to consider if there is a preferred moment of the day for reproduction. With an extremely changing environment that exposes animals to intense variation of environmental cycles and a peculiar life cycle which demands a high synchronization of social interactions, this species presents an unique opportunity to study the environmental and social synchronization of biological rhythms.

MATERIAL AND METHODS

Collection and maintenance conditions. Adult individuals of *Garcialebias reicherti*, male (n = 10, standard length = 4.35 cm, weight = 1.97 g) and female (n = 10; standard length = 2.75 cm, weight = 1.0 g) were collected with a hand net from temporary ponds located in Treinta y Tres, Uruguay, 32°58'56.89"S 53°52'13.02"W, in October 2015 and August 2016 to evaluate activity patterns in isolation and reproductive context, respectively. Length and weight were in the range reported for the species. Voucher specimens were deposited in the Fish Collection of Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay (ZVC-P 15708). Fish were kept in an indoor facility 15 days prior to activity trials under constant temperature (19ºC) and natural photoperiod (natural light through ample windows). Males were kept in individual aquariums (20 x 9 x 15 cm, length x width x height) to avoid harmful agonistic interactions, while females were kept in communal aquariums (40 x 13 x 15 cm) in groups of five individuals. Water was replaced every three days in a third of its volume. During both maintenance and trials fish were fed *Tubifex* sp., following a randomized schedule to avoid synchronization (Blanco-Vives, Sánchez-Vázquez, 2009; Sánchez *et al*., 2009).

Experimental procedures. The experimental setup consisted of aquariums (45 x 15 x 15 cm) divided transversely by external markings into three zones of equal size. To reduce any external disturbance and provide an uniform background, white screens were placed covering the walls of the aquarium. For continuous recordings, four infrared cameras (Protecta, model PTC-CI20B-65) connected to a digital video recorder were arranged for a top view of the aquarium. Fish were acclimated for five days to the experimental conditions before the beginning of the recordings. After completing the trials, all fish were kept as breeding stock.

Locomotor activity in isolation. To evaluate locomotor activity in isolation, females (n = 5) and males (n = 5) were individually placed in experimental aquariums with constant temperature (19°C) and a 13L:11D (13 h light: 11 h dark) photoperiod (lights on at 6:00), adjusted to the natural photoperiod at the moment of the experiment. Locomotor activity was recorded for eight days. Light intensity in the water surface during the light phase was 200 lux (consistent with values recorded in the natural habitat).

Locomotor activity and reproductive events in a reproductive context. To evaluate locomotor activity in reproductive context, dyads (n = 5) composed of a male and a female fish were placed in experimental aquariums with constant temperature (19°C) and a 12L:12D photoperiod (lights on at 09:00 h), adjusted to the natural photoperiod at the moment of the experiment. Locomotor activity was recorded for four days. Light intensity in the water surface during the light phase was 200 lux. In order to evaluate reproductive activity, each experimental aquarium was equipped with a circular container (12 x 3 cm, diameter x height) containing borosilicate pellets (Thomas Scientific beads 0.5 mm) as spawning substratum. Containers were placed at the center of the aquarium.

Data processing and statistical analysis. Video recordings were visually inspected to determine the locomotor activity during six days. Locomotor activity was measured as the number of crossings through the reference marks on the aquarium during the first 10 min of each hour. We calculated the daytime activity (summed diurnal activity of the eight days), night time activity (summed nocturnal activity of the eight days) and total activity (total locomotor activity of the whole trial period). In reproductive context trials, the locomotor activity of each individual of the dyad was quantified separately. An estimation of the reproductive activity was assessed by the recording of mating burials in the spawning substratum (García *et al*., 2008) during the first 10 min of each hour for the whole day.

In order to assess rhythmicity we performed a standardized chronobiological analysis consisting of the fitting of cosine function with a 24 h period on a time series containing locomotor activity data for each individual (Cornelissen, 2014). The time of maximum amplitude of the fitted curve (locomotor activity), known as acrophase, is the individual parameter used for statistical validation of rhythmicity among different experimental groups. Rayleigh test for circular statistics was used for testing rhythmicity among individuals while also calculating the mean acrophase value for the group. Circular statistics analysis (cosinor and Rayleigh) was performed using "El Temps" (Díez-Noguera, 1999) and *ad-hoc* Python routines (Van Rossum, Drake, 2009). Actograms (representation of locomotor activity variation in the time domain) were plotted for individual and paired fish. Locomotor activity synchronization in dyads was assessed by the Pearson correlation function.

The amount of locomotor activity was compared between the light and dark phases, sexes, and contexts. Data was checked for normality using Kolmogorov–Smirnov tests. We used nonparametric test (*i.e*., Wilcoxon signed-rank and Mann-Whitney tests), as data did not follow normality. Unless otherwise stated, reported values are mean ± standard error. Statistical analyses were performed using PAST software version 2.16. Differences were considered significant when $p < 0.05$.

RESULTS

Daily rhythm of individual locomotor activity. Locomotor activity in isolated individuals is mainly diurnal, although a certain amount of nocturnal activity is present. The Fig. 1 shows the analysis of daily rhythmicity as resulting from the individual actograms and cosinor analysis (Fig. 1A), followed by the Rayleigh test for circular statistics (Fig. 1B, see Tab. 1 for statistical summary). Representative actograms and cosinor diagrams of male and female fish (8 days, LD) show that locomotor activity is diurnal regardless of sex (individual actograms are shown in Fig. **S1**). Anticipation of lights-on for the onset of movement can be seen right before artificial sunrise in each of the plotted days. The average acrophase of males was at 18:06 (n = 5, Rayleigh test, p = 0.008) while that of females was at 17:44 (n = 5, Rayleigh test, p = 0.008). No sexual differences in acrophases were detected (male, n = 5 *vs*. female, n = 5; Mann-Whitney U test; p = 0.84), and so we performed a global analysis of daily rhythmicity that included all isolated fish. Locomotor activity shows maximum values in the peri-sunset hours (sunset at 19:00), in the range from 15:30 to 21:00, with a population acrophase (mean acrophase) at 17:55 (n = 10, Rayleigh test, $p = 2.7E-5$). This acrophase synchronization in isolated individuals responds to the stable phase locking of individual rhythms with the light/dark cycle.

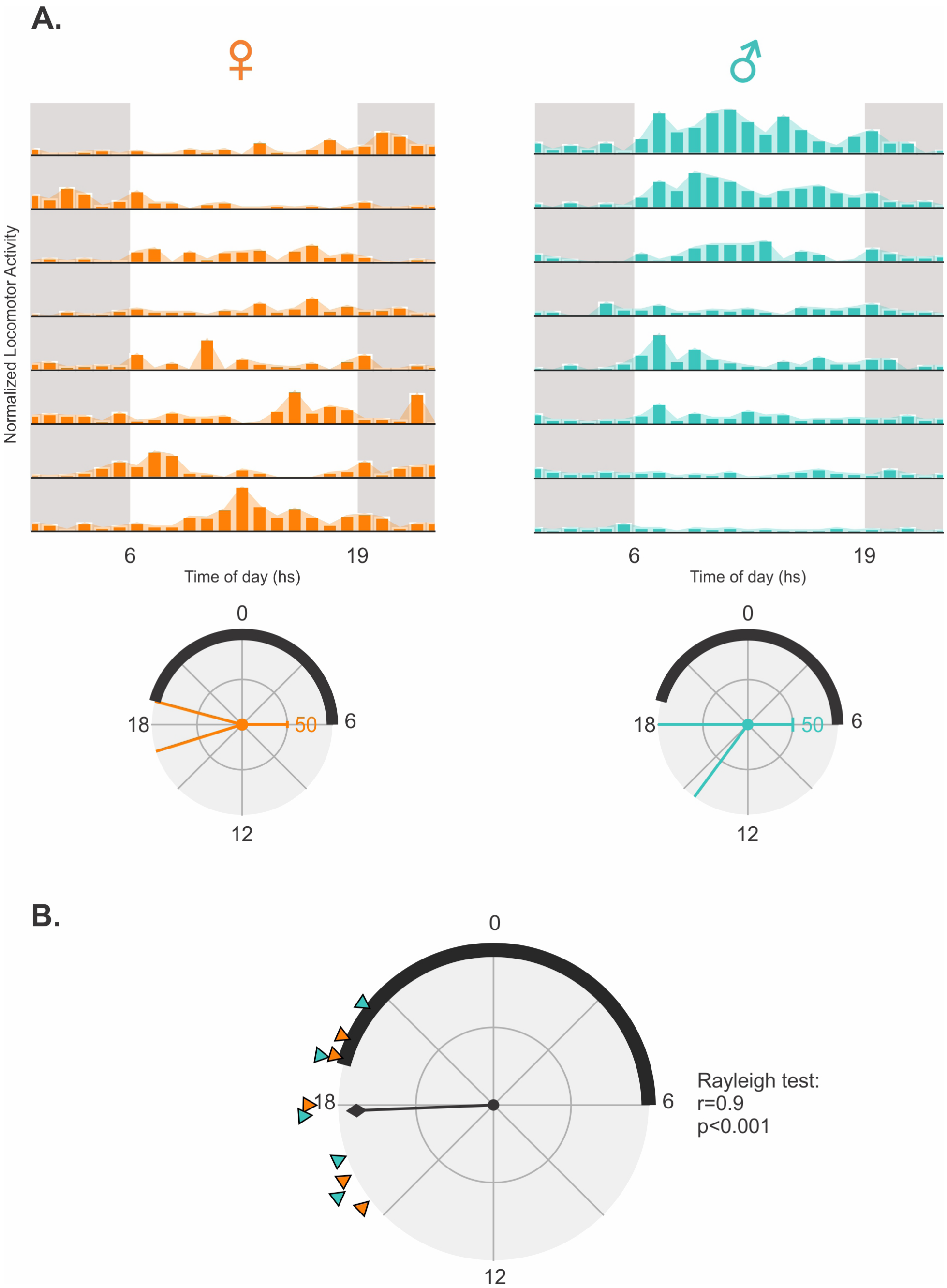

FIGURE 1 | Daily rhythm of locomotor activity in isolated fish of *Garcialebias reicherti* recorded during eight days in LD. **A.** Representative actograms and cosinor fit diagrams for a female (orange) and a male (green) fish. Amount of locomotor activity (number of events) is normalized for visualization purposes. Gray areas in the actogram represent the dark phase of each 24 h period. The cosinor representation for each individual is shown below the actogram. Black outlines represent the duration of the night. The internal circumference depicts the p = 0.05 confidence limit. Radial lines show the extreme values of the acrophases calculated for each of six days. **B.** Rayleigh test for all isolated individuals (5 males and 5 females) analyzed collectively. Triangles signal individual acrophases. The internal circumference depicts the p = 0.05 confidence limit and length of the black radial line marks the p value (external circumference is p = 0, see main text for p value). The black outline represents the duration of the night.

Daily rhythm of locomotor activity in reproductive context. In order to assess differences in locomotor activity induced by the reproductive context, female-male pairs housed in the same tank were recorded during four days. The Fig. 2 shows the analysis of the daily rhythms recorded in this condition. Superimposed actograms of two representative pairs in LD regime, and their respective individual cosinor diagrams, show that locomotor activity is allocated mainly during the day for both individuals of the pair (Fig. 2A, actograms for every pair are shown in Fig. S2). A global analysis of the locomotor activity of all reproductive individuals was performed using the Rayleigh test (Fig. 2B). Individual acrophases are close to sunset (19:00), and 8 out of 10 individual acrophases fall in a 3 h range from 18:00 to 21:00. The mean population acrophase occurs at 18:39 (n = 10, Rayleigh test, p = 1.30E-04). As in isolated fish, no sexual differences were detected in this analysis (male, n = 5 *vs*. female, n = 5; Mann-Whitney U test; p = 0.42). Furthermore, reproductive context had no effect on individual acrophases (Isolated, n = 10 *vs*. Reproductive, n = 10, Mann-Whitney U test; p = 0.28).

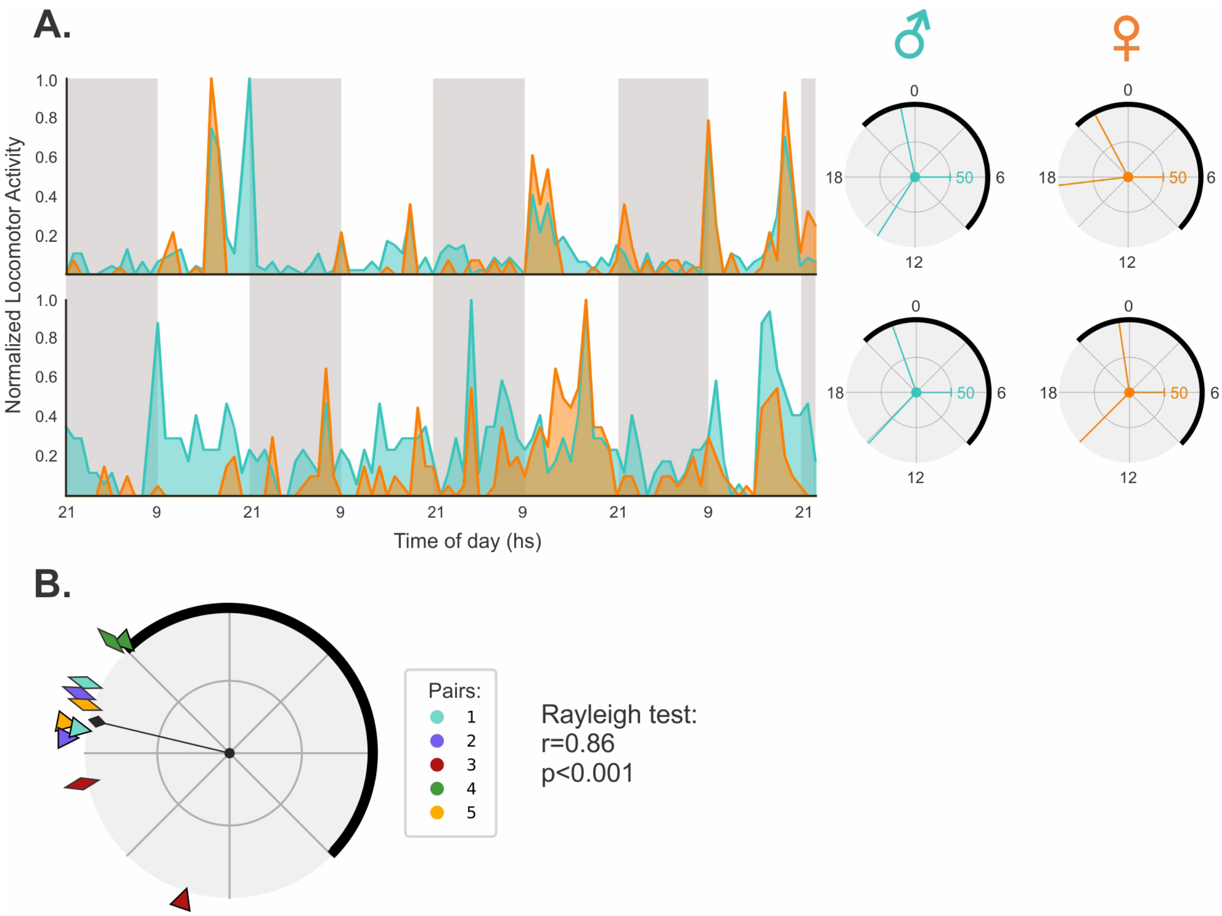

FIGURE 2 | Daily rhythm of locomotor activity in paired fish of *Garcialebias reicherti* recorded during four days in LD. **A.** Representative time series (actograms) and cosinor fit diagrams for two female (orange) and a male (green) dyads. Amount of locomotor activity (number of events) is normalized for visualization purposes. Gray areas in the actogram represent the dark phase of each 24 h period. The cosinor representation for each individual of the dyad is shown next to the actogram. Black outlines represent the duration of the night. The internal circumference depicts the p = 0.05 confidence limit. Radial lines show the extreme values of the acrophases calculated for each of six days. **B.** Rayleigh test for all paired individuals (5 males and 5 females) analyzed collectively. Triangles signal individual male acrophases, diamonds signal individual female acrophases. Members of each dyad are presented in the same color. The internal circumference depicts the p = 0.05 confidence limit and length of the black radial line marks the p value (external circumference is p = 0, see main text for p value). The black outline represents the duration of the night.

Locomotor activity in individuals of the same pair seems to be relatively synchronized, both in terms of the daily phase of the locomotor rhythm as well as in terms of the initiation of movement throughout the whole period of recording. The former is evident in the proximity of the acrophases among the two members of the pair, since in four out of five pairs, the difference between the individual acrophases was less than 30 min (Fig. 2B, members of the same pair presented in the same color). As for the latter, the coordination of locomotor events among the members of the couple was measured by cross-correlation analysis of the respective time series. The Tab. 1 shows Pearson's correlation values for the two individuals of the pair.

Influence of reproductive context on locomotor activity. Quantification and statistical analysis of locomotor activity according to sex, photoperiod phase (day *vs.* night) and reproductive context is presented in Tab. 2. Locomotor activity is higher during the day, regardless of sex and social context. Moreover, within each social context males and females show similar amounts of locomotor activity throughout the 24 h cycle. Although reproductive context had no effect on the rhythmicity of locomotor activity, it did have a modulatory effect on its magnitude (Fig. 3). A comparison between isolated and paired fish shows that the reproductive context induces a 40% decrease in the amount of locomotor activity (Isolated, n = 10 *vs*. Reproductive, n = 10, Mann-Whitney U test, p = 0.023).

TABLE 1 | Locomotor activity of *Garcialebias reicherti* in relation to sex (males *vs.* females), social context (isolated, i *vs.* reproductive, r) and light/dark cycle phase (day *vs.* night). Values are shown as mean ± standard dev. (range between parentheses). P values of the respective Mann-Whitney U test are shown, and statistical significance is marked with an asterisk. Male (i) = isolated males; Female (i) = isolated females; Male (r) = males in reproductive context; Female (r) = females in reproductive context. Comparisons: All (i) = comparison among all isolated fish for each phase (day or night); All (r) = comparison among all reproductive fish for each phase (day or night).

	Male (i)	Female (i)	p	Male (r)	Female (r)	p	All (i)	All (r)	p
			Male (i) *vs.* female (i)			Male (r) *vs.* female (r)			Isolated *vs.* reproductive
Day	829.4±356.6 (496-1412)	714.2±334.4 (395-1246)	0.530	534.6±268.9 (309-992)	401.2±245.9 (206-826)	0.146	728.5±331.5 (395-1412)	393±252.9 (206-992)	0.022*
Night	320.4±76.7 (197-395)	515.4±336.2 (124-993)	0.532	263.2±44.1 (214-319)	213.6±132.1 (88-376)	0.680	353.5±251.8 (124-993)	244.5±96.4 (88-376)	0.053*
p	0.043*	0.043*		0.043*	0.043*		0.005*	0.005*	

TABLE 2 | Correlation indexes (r) of locomotor activities among the individuals of each dyad of *Garcialebias reicherti*. Asterisks signal statistical significance for each r value (* = p<0.001).

Dyad	r
1	0.7*
2	0.56*
3	0.49*
4	0.37*
5	0.47*

Timming of reproductive events. Of the five couples analyzed, four showed reproductive activity. Reproductive events are conspicuous and have been extensively described previously. Event occurrence throughout the day was analyzed and is presented in Fig. 4. Reproductive activity is strictly diurnal covering a range from 9:00 to 19:00 with more than 70% of the events occurring before midday.

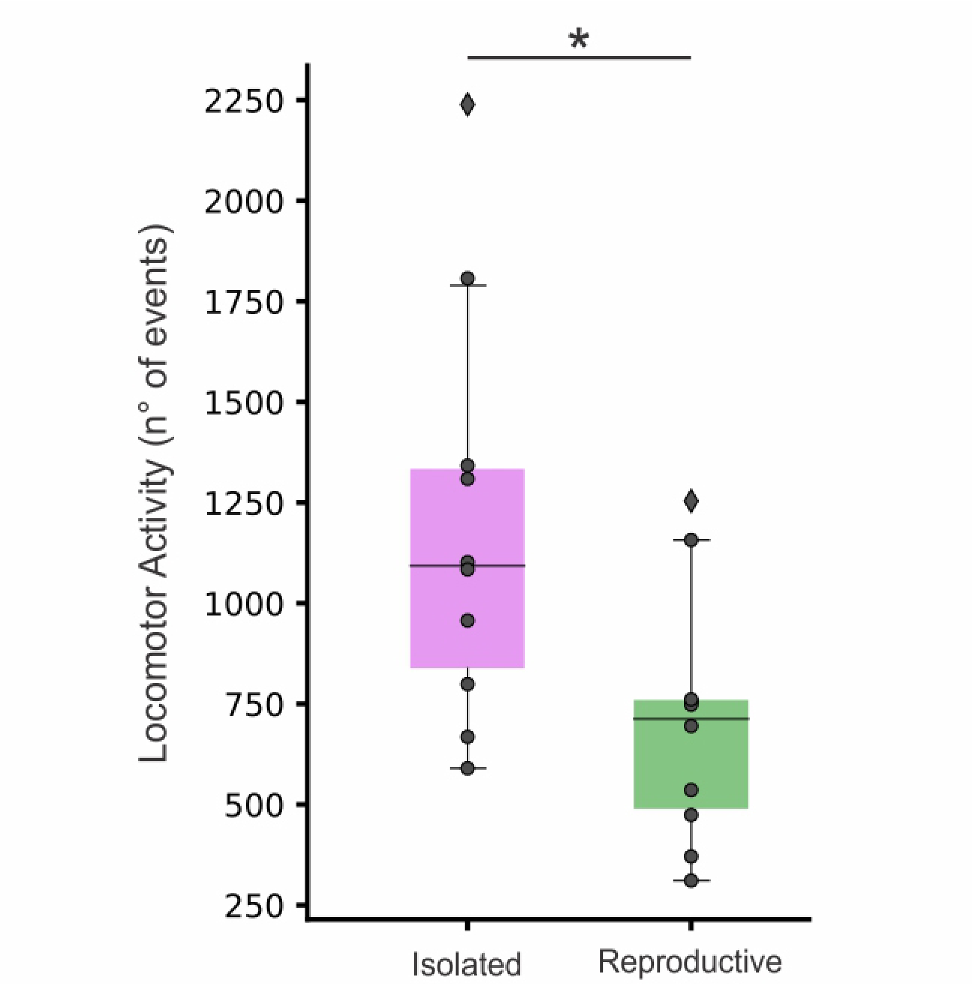

FIGURE 3 | Contextual modulation of locomotor activity of *Garcialebias reicherti*. Total daily locomotor activity for isolated (n = 10) and paired fish (n = 10). Each dot represents the mean number of events for each fish. *shows statistical significance (see p value in the main text). Box height from upper to lower quartile, whiskers represent standard deviation, median shown by horizontal line.

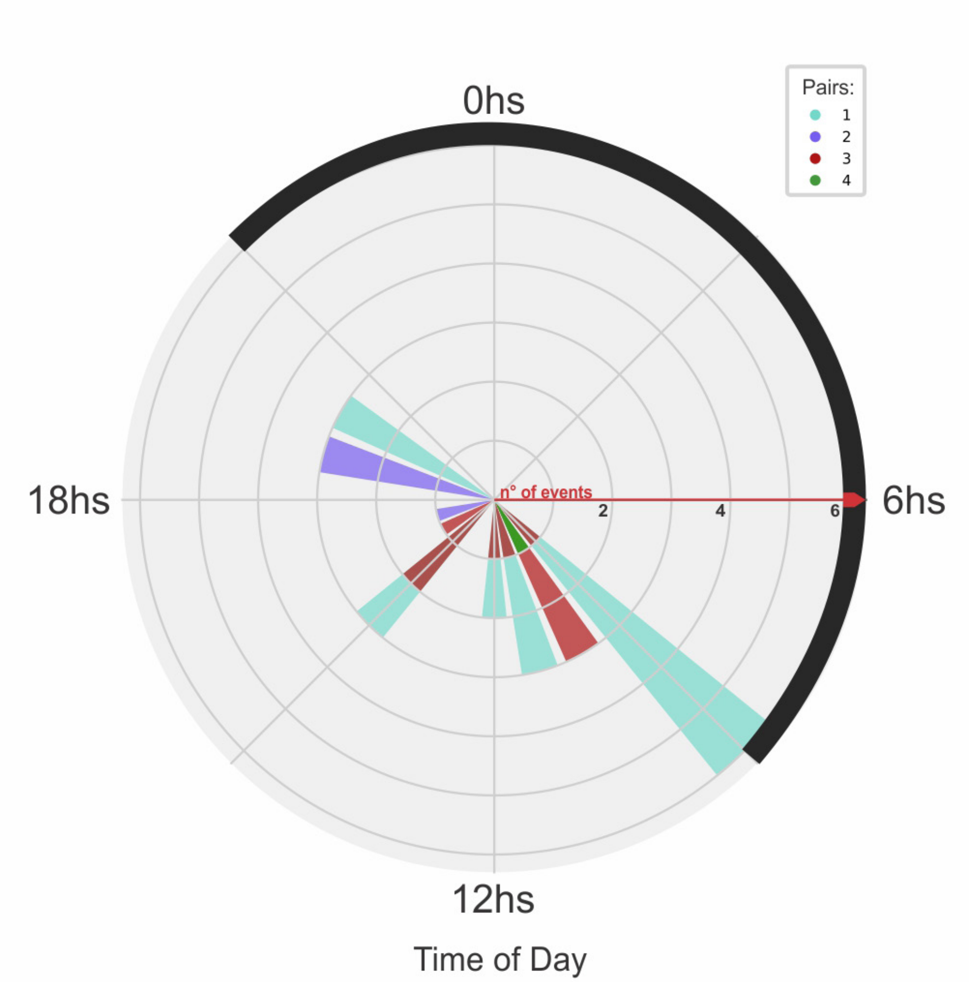

FIGURE 4 | Number and allocation of reproductive events at different hours in *Garcialebias reicherti*. Bars show the occurrence of events at different timepoints for each dyad (see references). Concentric circumferences show the number of events at that hour throughout the 4-day period. Black outline signals the duration of the night.

DISCUSSION

In this work we show that *Garcialebias reicherti* is a diurnal species, as evidenced by the synchronization of the daily pattern of locomotor and reproductive activity with the light phase of the photoperiod. Moreover, we present evidence that a reproductive context modulates the pattern of diurnal locomotor activity promoting a decrease in total activity and the synchronization among the individuals of the pair.

Locomotor activity. Locomotor activity is higher during the light phase of the day. This holds true for both isolated and reproductive animals. Similar results have been reported for African annual fish of the genus *Nothobranchius* (Haas, 1976; Lucas-Sánchez *et al.*, 2011, 2015). However, while *Nothobranchius* have acrophases close to midday, *G. reicherti* maximum locomotor activity is allocated closer to sunset. It is worth noticing that in the reports for *Nothobranchius* fish were routinely fed at a fixed time (midday). Feeding regimen is a potent *zeitgeber* which could be entraining locomotor activity and hence influencing acrophases (Mistlberger, 1993; Singh *et al*., 2016). Since the mean population acrophase is close to sunset it could be interesting to consider whether locomotor activity is diurnal or crepuscular. The term crepuscular refers to activity with maximum expression coinciding with twilight, the peri-sunrise and peri-sunset period characterized by an extremely dim light which occurs when the sun is between zero degrees (sunrise/sunset) and 18° below the horizon (Potts, 1990). Our experimental laboratory protocol, with lights on at 6:00 and off at 19:00 does not include a twilight period. In this sense we can not conclude that this rhythm is crepuscular. Instead, we conclude they are diurnal, since the mean population acrophase is diurnal (17:55). Locomotor activity recordings in the natural habitat (when animals are actually exposed to twilight) would be necessary and extremely interesting, to determine crepuscularity

The proportion of activity occurring during the day and the acrophase of locomotor rhythms are similar in both isolated and reproductive animals, showing a robust diurnal behavior. This diurnality is particularly advantageous for annual fish, as they possess strong visual acuity supported by the anatomical development of their visual system as well as extensive neural proliferation associated with visual structures (Casanova *et al*., 2015; Berrosteguieta *et al*., 2022). These characteristics suggest their reliance on visual information for various behavioral displays, such as the intensification of body pigmentation associated with courtship and hierarchical position (Passos *et al*., 2013), as well as locomotor displays and fin placement linked to courtship and aggression. Additionally, sympatric species differ exclusively in male pigmentation, indicating that visual information plays a critical role in species recognition and reproductive isolation (García *et al*., 2008; Passos *et al*., 2016). Mechanosensory and chemical information are also relevant and most likely participating in perception. For instance, in a hybridization context, females tell apart conspecific mates from heterospecific mates based upon chemical cues (Reyes-Blengini *et al*., 2018). The advantage of diurnality becomes particularly evident, especially at the beginning of the reproductive season when water is clear (Passos *et al*., 2021). The visual clarity provided by daylight facilitates the effective use of visual information for species recognition, courtship, and other social interactions. This opens an interesting question regarding changes in relative amounts of diurnal *vs*. nocturnal locomotor activity associated with changes in water turbidity, as a proxy of seasonal changes.

The impact of the reproductive context. Social reproductive context promotes two clear modulations on activity patterns: i) a decrease in the total amount of locomotor activity and ii) the synchronization of locomotor events among individuals sharing the same tank. Isolation of gregarious animals promotes stress as evidenced by the physiological and behavioral changes reported in mammals (Donovan *et al*., 2020, 2022), birds (Apfelbeck, Raess, 2008) and fish (Tunback, 2020). Stress is commonly associated with cortisol release, which has been linked to elevated levels of locomotor activity (Øverli *et al*., 2002). Moreover, a peak in blood cortisol concentration anticipates or coincides with the onset of daily activity in mammals (Mohawk, Lee, 2005; Passos *et al*., 2021) and other animal groups including fish (Oliveira *et al*., 2013). Increases in locomotor activity in isolated animals as different as birds or fruit-flies, have been previously reported (Apfelbeck, Raess, 2008; Lone, Sharma, 2011; Tunbak *et al*., 2020) and linked to stress induced by social deprivation (Mumtaz *et al*., 2018). In *G. reicherti*, reproductive behavior and stress are linked, as increased cortisol levels resulting from environmental stressors actually promote reproduction (Passos *et al*., 2021). Therefore, isolated animals, with elevated cortisol levels induced by stress, might be actively exploring the environment, driven by the necessity to reproduce. Although not gregarious, *G. reicherti* inhabits densely populated ponds and, throughout its short life cycle individuals engage in intense reproductive activity from sexual maturation until death (García *et al*., 2008). Males are territorial and frequently engage in aggressive encounters in defense of oviposition sites. Isolation is a very rare condition for the species (Passos, 2013). When animals are housed in male-female pairs, the need for extensive exploration to find a mate for reproduction is minimized. Furthermore, our results suggest a strong correlation between locomotor events in the two individuals of a pair. Actograms presented in Fig. 2 show superposition of locomotor events between the paired fish, particularly during periods of intense activity. This is further supported by the high correlation indexes for the time series containing locomotor activity data of both members of a pair. This coordination is also confirmed by cosinor and Rayleigh analysis, showing that even though the individual acrophases span a range of three hours, the difference between acrophases of the two members of a pair does not exceed 30 min.

Reproductive behavior. *Garcialebias reicherti* has a single reproductive season extending from sexual maturity to death. Our results show that reproductive events occurred mainly during the first four hours of the day, similarly to what has been reported for African annual fish (Vrtílek, Reichard, 2016). The fact that reproductive events occur during the day is indicative of the importance of visual communication for reproductive behavior in relation to morphological characters and behavioral displays.

Social context is emerging as a significant modulator of rhythmic behavior in the field of chronobiology, even rivaling the influence of light/dark cycle (Tomotani *et al*., 2016; Migliaro *et al*., 2018; Gascue *et al*., 2020; Siehler *et al*., 2021). We show here that pair housing does not modify the acrophase for locomotor activity, which is associated with the last hours of the day for both isolated and socially housed individuals. However, behavior displayed in reproductive context brings to light the modulatory effect of this particular social context on the rhythm of locomotor behavior. The daily rhythm of reproductive events evidences the coexistence of two rhythmic processes, a reproductive rhythmic behavior occurring in the morning, and the daily variation of locomotor activity which has maximum values towards the evening. These rhythms must coexist in a coordinated expression in nature, while keeping a synchronization with environmental variables. Contextual information, especially socially relevant settings appear to play a fundamental role in the fine tuning of circadian rhythms, particularly in the expression of adaptive social behavior (Davison, Menaker, 2003; Favreu *et al*., 2009; Bloch, 2010).

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AUTHORS' CONTRIBUTION

Andres Olivera: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing-original draft.
Carlos Passos: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing-original draft.
Juan I. Vazquez: Formal analysis, Visualization, Writing-review and editing.
Bettina Tassino: Funding acquisition, Project administration, Validation, Writing-original draft.
Adriana Migliaro: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing.

ETHICAL STATEMENT

Collection and experimental procedures were approved by the ethical committee of Universidad de la República, Uruguay (240011-000511-17-CEUA-Udelar).

COMPETING INTERESTS

The author declares no competing interests.

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