Effects of social position on subsequent courtship and mating activity in an African cichlid

Anastasia Martashvili[1], Sara Jedwab[2], Lakshita Vij[2], Alvarado S.G.[1,2]

1. **Cognitive Neuroscience Program, Graduate Center of City University of New York, New York, NY 10016**
2. **Department of Biology, Queens College City University of New York, 65-30 Kissena Blvd, Flushing, NY 11361, USA**

*Corresponding author: sebastian.alvarado@qc.cuny.edu

Abstract
Within social hierarchies, rank can be dynamic and modulated by changes in molecular and/or physiological substrates. These changes can shape various behavioral outcomes that lead to the reorganization of hierarchies and their communities. Here, we sought to better understand how social environment and rank shape male spawning behaviors and outcomes in African cichlid fish *Astatotilapia burtoni.* First, using a social dyad paradigm, we generated territorial (T)/ Non-territorial (NT) male pairs. After establishing the social hierarchy, the behaviors of the Ts and NTs were recorded and scored. Afterward, pairs were separated and individually moved into a spawning phase, which consisted of a new tank with novel females and no other males where their behaviors were scored. While previous studies have shown how territorial/non-territorial/ascending/descending males have unique behavioral profiles, we sought to deepen this interpretation with a focus on the latency of decision-making and transition matrices representing enriched sequences of behavior. We found that while the courtship behaviors are shared between stably territorial and ascending males in the spawning phase, only the animals that were territorial in the dyad phase were the ones that succeeded in being reproductive in the subsequent spawning phase.

Keywords
Behavior, Social Behavior, Sociobiology, Cichlid, *Astatotilapia burtoni*

Statements and Declarations
Competing Interests: Authors have no financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

Acknowledgments:
We would like to thank funds provided by the Queens College Foundation, National Science Foundation (Award # 1921773), and The Professional Staff Congress of the City University of New York (TRADA-50-228) for funding provided to SGA, LV, and SJ and The Cognitive Neuroscience Master's program at the Graduate Center which supported AM. Special thanks to Andrew Claros who assisted with the development of these tools for behavioral analysis. Additional thanks to Dr. Maral Tajerian for revising this manuscript and providing constructive feedback on its writing.

Compliance with Ethical Standards
This research was approved by the Queens College Institutional Animal Care and Use Committee Protocol #188

Competing Interests
The authors have no competing interests to disclose.

Data Availability
Raw score logs of our data are readily available upon request.

Introduction

Social hierarchies are prevalent across the animal kingdom and allow individuals within a community to secure resources and mating opportunities. For example, social rank can bestow reproductive potential on an individual, which can lead to variation in behavior, physiology, and gross anatomy. These systems can be developmentally biased or plastic(Laland et al., 2015). For instance, the developmental constraints on reproduction in hymenopterans create a developmental bias that physiologically generates workers who cannot produce fertile offspring in the presence of a queen(Khila & Abouheif, 2008). Similarly, subsequent pregnancies in naked mole rats can lead to vertebrae elongation, leading to larger litters and queen caste determination(Dengler-Crish & Catania, 2009; Thorley et al., 2018). In contrast, social systems that are plastic can shift rank among conspecifics via behavioral transitions. For example, changes in behavior and serotonergic tone or gonadotropin function can shift ranks among Anolis lizards(Summers et al., 2005) and African cichlids(Maruska & Fernald, 2018), respectively. While changes in rank can present social opportunities, little is known about how transitions in rank can affect decision-making and complex behavior within a community. For example, how does previous social life history inform changes in a novel social environment, and does a continuum exist between developmentally plastic and biased behaviors?

Here, we adopted the African cichlid system, *Astatotilapia burtoni*, for its robust social hierarchy. *A. burtoni* are endemic to Lake Tanganyika and demonstrate plasticity in their behavior and morphology in response to their environment. *A. burtoni* has been recognized as an emerging model for the study of social behavior, which is an important factor that has shaped the diversity of cichlids in East African Great Rift Lakes. Social status within lek-like communities shows variation in male-specific coloration and social behaviors and is believed to be a consequence of intense sexual selection and male-male competition (Seehausen & Schluter, 2004). In the wild, *A. burtoni* have adopted lek-like social systems, wherein two distinct types of males are observed: territorial (T) and non-territorial (NT)(Fernald & Hirata, 1977) and females that shoal to visit leks for mating. T males tend to exhibit aggressive defense of specific areas within the habitat, typically against intruding males, whereas NT males refrain from establishing or defending specific territories. T males are commonly larger and display more vibrant coloration than their NT counterparts, which do not. Furthermore, these hierarchies and male stereotype behaviors can be replicated in semi-natural environments in the laboratory (Fernald, 1977).

In the laboratory, the behavioral dynamics of *A. burtoni* males regarding territoriality have been extensively studied in relation to the neurobiology of social behavior (Maruska & Fernald, 2018). Male *A. burtoni* fish naturally assume territorial status within their environment(Fernald, 1980) even in the absence of competitors, suggesting that their territorial behavioral displays are what suppress non-territorial males from ascending in rank. Changes in rank via a social ascent or descent result in rapid shifts in behavior within minutes(Maruska et al., 2013; Fialkowski et al., 2021). For example, the removal of a dominant male from a tank with suppressed NT males presents the social opportunity to ascend to territorial status(Maruska, 2015). Similarly, T→NT transitions are conjoined with bidirectional changes in behavior that are accompanied by rapid changes in cortisol and immediate early gene expression in the brain(Maruska et al., 2013). Additionally, changes to the physical environment can prime transitions in social ascent/descent via the modulation of growth rates (Hofmann et al., 1999) or morphological coloration(Fang et al., 2022) and their accompanying behavioral profiles(Korzan et al., 2008). This suggests that the physical environment can have profound effects on their social environment.

Given the broad developmental plasticity seen in *A. burtoni*, we adopted the system to ask the question: how do stable and transitioning ranks shape the reproductive behaviors of males? We present a study of T/NT males generated by a stable dyad paradigm and subsequent exposure to a novel social environment where a focal male can mate with multiple females. Briefly, T/NT male pairs were generated over a 1-month dyad and put into a larger spawning paradigm consisting of a larger tank with ten females where the behavior of the focal male was scored in the first hour. We sought to investigate whether their past experiences in a stable dyad T/NT male would affect their behavioral patterns as territorial individuals in a novel social environment (spawning phase). In addition to

examining the incidence of different behaviors, we complement our understanding of these behavioral profiles with analyses of the latency to make decisions and behavioral sequence transitions to identify nuanced differences between behavioral profiles. We anticipate a deeper understanding of these processes will inform our understanding of male social behaviors, how they shape their fitness and the evolution of behavior in cichlids.

Methods

Animals and animal husbandry

Subjects were laboratory-bred cichlid fish *A. burtoni*, derived from stock wild-caught in Northern Lake Tanganyika, Africa(Fernald & Hirata, 1977). All animals in this experiment were bred and reared in community tanks set up to mimic the natural conditions of Lake Tanganyika, of pH 8, 25°C, flow down system with water change twice a day, salt 280 ppm, a 12-hour day/night cycle (Alvarado et al., 2015a). They were fed once a day at 9 AM and kept in brown gravel-lined tanks. At the time of the experiment, all animals were six months to one year old and reared in community tanks (~40 males and females in 115 L tanks), and all females were adults capable of spawning as they all had at least one brood previously.

Experimental setup and behavioral recording

In the social dyad phase of the experiment, two males were size-matched and housed with three females for one month (N=4) (Fig.I). The dyad tank had a natural substrate comprising brown gravel and half of a terracotta pot functioning as a territory (Fernald, 1977). At the end of one month, dyad tanks were filmed for one hour with cameras (GoPro Hero8 Black) mounted on tripods (Amazon Basics WT3540). In the second phase of the experiment, directly after the dyad filming concluded, each male was removed from the dyad tanks and put separately into the spawning phase: a new tank with brown gravel, half of a terracotta pot, and ten adult females the focal male had never interacted with before. Filming started immediately, and they were filmed for one hour. Following 16 hours after the filming, mouthbrooding females were counted in each tank. Spawning trials were staggered, with subsequent spawning phases taking place with alternating males of different ranks.

Behavioral Scoring and Analysis

Videos were scored in BORIS event-logging scoring software(Friard & Gamba, 2016) by a blinded scorer using an ethogram previously described(Fernald & Hirata, 1977; Friard & Gamba, 2016). Score logs were exported from BORIS in a CSV format. Base R in RStudio-2023.12.1-402 was used to prepare the data for transition matrices(Gandrud, 2013). After combining Dyad and Spawn data into one data frame using Base R, male-oriented behaviors were removed to account for the spawn phase, only having a focal male to prepare the data for comparative analysis. To perform paired t-tests on individual behaviors, it was necessary to add zero values for behaviors that were not observed by some individuals. This was done with the package tidyr. Next, relative frequencies were calculated, normalizing behaviors within each subject. Additionally, to assign behavioral categories to each behavior, package diplyr was used. Package Stats was used to run paired t-tests on individual behaviors and behavioral categories, comparing the Dyad T with Dyad NT and Spawn T with Spawn NT, respectively. The Benjamini-Hochberg correction, also known as false discovery rate (FDR) correction, was utilized to adjust for the increased likelihood of false positives when conducting multiple hypothesis tests simultaneously, using the p.adjust() function in R(James et al., 2023). Box plots conveying the results were generated with ggplot2. For the latency graphs, we created a data frame using base R that reflected the behavior pair and the time taken to transition from one behavior to another, mean, and standard error of the mean (SEM) of each behavior done by each subject in each phase. Utilizing ggplot2, this data frame was visualized with a scatter plot. Package Stats was utilized to run paired and independent t-tests to compare the decision making latencies within individuals and across assays.

Python version 3.11.5 and its libraries such as NumPy, pandas, NetworkX, and Matplotlib were used to generate Markov chains. The data was arranged by looping through the CSV data frames and extracting just the behavior values, tank IDs, and time stamps. Through utilizing the itertools package, we created pairs of all possible permutations of behaviors, including behaviors becoming pairs within themselves (Foundation, 2023). Then, we utilized two functions: one to increment the individual behavior based on its frequency and the second to increment the pair based on their frequency. By counting the occurrence of each behavior type alongside how many times a particular behavior succeeded it and then dividing the latter by the former, the probability of one behavior occurring right after the other was calculated. Using this, along with the Python packages Networkx and Matplotlib, we developed nodes representing each behavior, along with their relative sizes reflecting how frequently each behavior occurred. The thickness of the connecting lines between nodes showcases how likely one behavior is to succeed another.

All figures were finalized using Biorender.

Results

Comparative Analysis of Behavioral Frequencies between dyad and spawning paradigms

In the dyad phase, Ts did significantly more pot-related behaviors such as *"pot entry"* ($Mean_T$=0.059±0.006, $Mean_{NT}$=0, $p<0.01$) and *"pot exit"* ($Mean_T$=0.057±0.005, $Mean_{NT}$=0, $p<0.01$)(Fig. II). In contrast, NTs did more "*flee from female*"($Mean_T$=0, $Mean_{NT}$=0.162±0.043, $p<0.05$). When considering indices for aversive, reproductive, and aggressive categories, Ts did more aggressive behaviors ($Mean_T$=0.066±0.021, $Mean_{NT}$=0.005±0.002, $p<0.01$), and NTs engaged in more aversive behaviors ($Mean_T$=0.001±0.001, $Mean_{NT}$=0.300±0.089 $p<0.05$). In terms of behavioral frequency changes for each individual across dyad and spawn phases, NTs engaged in significantly more *"pot entry"* ($Mean_{Dyad}$=0, $Mean_{Spawn}$=0.119±0.017, $p<0.01$) and *"pot exit"* ($Mean_{Dyad}$=0, $Mean_{Spawn}$=0.119±0.016, $p<0.01$) in the spawning phase than in dyad phase.

Comparative Analysis of Behavioral Transitional Probabilities between Dyad and Spawning paradigms

In the dyad phase, NTs are 89% more likely to transition from *"chase male"* to *"attack male"*, however, *"chase male"* is only involved in 2 different behavioral combinations accounting for only 0.3% of total behaviors done by the NTs and *"attack male"* is involved in 5 behavioral combinations and happens only 1% of the time. Moreso, NTs are 25% more likely to *"attack male"* and then *"flee from male"*, but NTs only attack the Ts 1% of the time. It is 13% more likely that NTs will follow up *"chase female"* with *"flee from male"*, but the actual behavior of "*chase female"* is only done by the NT 2% of the time. NTs are also 37% more likely to transition from *"attack male"* to *"attack female"* than T males. While NTs do not attack Ts often (1%), *"attack female"* is involved in 10 different behavior combinations done by NTs and it accounts for 37% of the total behavior, being second most commonly observed, on par with territorial males (19 combinations, 36%). Therefore, even though these particular transitions are more likely to happen for the NTs than Ts, the actual incidence of these behaviors is low.
The most common behavior observed by the NTs is *"flee from male"*, accounting for 47% of total behavior and 10 different behavioral combinations. The following behaviors done by NTs lead to them fleeing from Ts: *"attack female"* (Probability=30%), *"flee from female"* (Probability=50%), *"chase female"* (Probability=13%), *"attack male"* (Probability=25%), *"flee from male"* (Probability=62%) and *"dig"* (Probability=7%). Important to note that frequency-wise, NT males do not engage in behaviors of *"dig"* (1% of total behavior), *"chase female"* (2%) and *"attack male"*(1%). Collectively, *"attack female"*, *"flee from female"* and *"flee from male"* are the three main behaviors and are most likely to lead to Ts chasing after NTs and NTs fleeing from Ts.

The third most common behavior for the NTs is *"flee from female"*, which accounts for 11% of the total behavior and is involved in 6 different transitional combinations. There is a 50% chance, this behavior is followed up with

"flee from male", 26% chance for *"attack female"*, 21% for *"flee from female"*, and 2% for *"attack male"*. Three behaviors are likely to precede NT's *"flee from female"*: *"attack female"* (9%), *"flee from male"* (12%) or *"flee from female"* (21%).

For Ts, the most frequent behavior is *"attack female"*, which accounts for 36% of total T behaviors and is involved in 19 behavioral combinations. Considering a 10% threshold for probabilities and behavioral frequencies, if the initial behavior is *"attack female"*, Ts are 48% likely to follow with *"attack female"* again, 23% likely to *"chase female"* and 10% likely to *"attack male"*. Furthermore, Ts are 16% likely to follow up *"dig"* with *"attack female"*. The second most frequent behavior is *"chase female"*, which is 21% of total behavior and part of 15 combinations. If T engages in *"chase female"*, there is a 39% chance it will lead to *"attack female"* and a 39% chance it will lead to *"chase female"* again. The third most common behavior done by Ts is *"dig"* at 17% and 17 combinations. There is a 58% chance that T will *"dig"* after *"dig"*, and 16% chance that T will *"attack female"* after *"dig"*.

We identified differences in behavioral sequences and transitional probabilities between the Ts and NTs in the subsequent spawning phase (Fig.III). The sum of absolute differences (SAD), which is a measure used to quantify the difference between two sets of data points, was used to further study the differences in probabilities of behavioral transitions between T and NT males. Nine behavioral transitions in total fell over the 10% threshold consideration. Territorial males were 12% more likely to transition from *"chase female"* to *"pot entry"* and 17% more likely to go from *"lead swim"* to *"pot entry"* than NT males. Conversely, NT males were approximately 10% more likely to transition from *"pot exit"* to *"attack female"*, *"chase female"* to *"chase female",* and *"lead swim"* to *"chase female"*. Also, NTs were 13% more likely to go from *"lead swim"* to *"lead swim"*, 14% more likely to *"quiver at female"* and then *"flee from female"*, 15% more likely to *"flee from female"* and transition to *"attack female"* and 20% more likely to *"quiver at female"* and then *"chase the female"*.

10 behavioral transitions were performed only by the territorial males in the spawning phase (see Supplemental Table 2). Of those transitions, 4 were equal to or over the 10% threshold. Ts were 10% likely to transition from *"quiver at female"* to *"quiver at female"* and to go from *"quiver at female"* to *"lead swim"*. Ts were also 50% likely to transition from *"lateral display"* to *"dig"* and from *"lateral display"* to *"attack female"*. Furthermore, four transitions were only performed by previously non-territorial males, however, only one fell over the 10% probability threshold: previously NT males were 14% likely to transition from *"quiver at female"* to *"flee from female"*(see Supplemental Table 2). Keeping in mind which behaviors are most frequently observed (threshold of 10% of total behaviors), Ts are mostly engaging with *"attack female"*, *"pot entry"*, *"pot exit"* and *"quiver female"* while NTs are mostly doing *"attack female"*, *"pot exit"*, *"pot entry"* and *"chase female"*. Ts were more likely to transition between more combinations of behaviors than NTs, however, despite some differences, the behavior repertoires of Ts and previously NTs are similar. Moreso, there is a drastic difference between the transitional probabilities and behavioral repertoire between the dyad and spawn phase NT subjects. NTs go from barely engaging in any behaviors or transitions to almost the same behavioral repertoire as the T males in the spawning paradigm.

Comparative Analysis of Latency of behavior initiation and Behavioral Transition Decision making in Spawning Phase

In the spawning phase, there was no statistical difference in the latency of any behavior initiations between T and previously NT males (see Supplemental Figure 1). Of note, NTs did not perform *"lateral display"*, while Ts did so twice. Furthermore, the time taken to transition between two behaviors by Ts and NTs was analyzed. In the dyad phase, NTs took significantly longer to transition from *"attack female"* to *"attack female"* compared to Ts ($Mean_T$=4.30 ±0.25, $Mean_{NT}$= 17.61±3.50, $p<0.01$) (see Supplemental Figure 2a) In the spawning phase, there weren't any differences in decision-making latency between T and NT males (see Supplemental Figure 2b) When comparing the decision-making time of the same subjects across phases, we find that Ts, when they are in dyad

phase, are much faster to transition from *"attack female"* to *"attack female"* ($Mean_{Dyad}$=4.30±0.25, $Mean_{Spawn}$=9.72±1.55, P<0.05) and from *"attack female"* to *"chase female"* ($Mean_{Dyad}$=4.28±0.38, $Mean_{Spawn}$=15.11±3.30, P<0.05) compared to when they were in spawn phase (see Supplemental Figure 2c). Conversely, in the dyad phase, NTs were much slower to transition from *"attack female"* to *"attack female"* compared to the spawn phase ($Mean_{Dyad}$=17.61±0.50, $Mean_{Spawn}$=5.60±0.68, P<0.01)(see Supplemental Figure 2d). Important to note that in the dyad phase, NTs engaged in fewer behaviors in general, therefore, there were behavioral transitions that occurred at very low incidence and that data was insufficient for the paired-t test analysis. All of the territorial males reproduced (1-2 broods), however, none of the previously non-territorial males were successful in reproduction.

Discussion

This study presents territorial and non-territorial behavior sequences within a dyad and follows a change into a novel social environment in the form of a spawning paradigm. While the courtship behaviors are shared between these two groups in the spawning phase, only the animals that were territorial in the dyad phase succeeded in being reproductive in the subsequent spawn phase. When presented with a social opportunity, behaviors associated with territoriality in ascending NT males were reinstated, provided the social opportunity consistent with previous reports (Maruska & Fernald, 2010). Our findings remain consistent with the behaviors that occur during a social ascent in *A burtoni*, along with the inclusion of decision-making latencies and behavioral sequences that illustrate the similarities between T and ascending NT males.

In the spawning phase, T and NT transitional probabilities are similar. However, Ts exhibit more complexity in transitions, which could be the result of the novelty of the environment and females (Fig.III), which may be analogous to social novelty behaviors reported in other teleost systems such as zebrafish(Barba-Escobedo & Gould, 2012; Nascimento & Maximino, 2023).

In the spawning phase, there are behavioral transitions that seem to be subject-specific (see Supplemental Table 2). The behavioral transitions exhibited solely by the territorial male fish depict a complex interplay of aggression, courtship, and territorial defense. "*lateral display*" is connected to "*attack female*," "*lead swim*," and "*dig*." It is possible that through "*lateral display*" (aggressive posturing), the male may be attempting to assert dominance over the female. "*lead swim*" to "*lateral display*" could indicate a failed attempt at courtship leading to renewed aggression. However, "*dig*" serves as a function of territory upkeep, which is important for courtship because that territory is also the spawning area. It is possible that "*lateral display*" was used by the Ts to defend and upkeep the territory from females with whom they failed courtship. It is important to note that "*lateral display*" occurred rarely for both Ts and NTs. Previous work has suggested that behavioral patterns involving "*lateral display*" (including "*pot entry*" and border fights) may be tied to circadian behaviors occurring more often in the afternoon than in the morning (Alward et al., 2019), underlining the importance of a longer study window for studying behavioral variation.

The behavioral transitions observed in NT fish, where they move from "*quiver at female*" to "*flee from female*," may suggest a reversal from courtship to avoidance behavior, possibly triggered by the female's rejection or aggression towards the male's advances. It is important to note that transition matrix analysis, like other probability measures, has a shortfall in that it only accounts for incidence and not duration. Intuitively, it is easy to consider that subjects spend more time performing behaviors that they are more likely to engage in, but additional behavioral experiments need to be conducted to confirm this. Previous studies have looked at behavior patterns of stably territorial and ascending males and found that ascending males exhibited different patterns of aggressive behavior compared to stable T males (Maruska & Fernald, 2010; Alward et al., 2019). Specifically, ascending males were less likely to follow aggression with courtship compared to territorial males. However, this distinction diminished when longer intervals between actions were considered, suggesting that ascending males can adopt typical territorial behaviors

but with slower transitions from aggression toward courtship. In our study, however, there was no difference in the transition latency of any behaviors between T and previously-NT males (See Supplemental Figure 2).

Behaviors involved in the highest number of combinations for each subject may suggest the subject's tentative motivations. For Ts, *"attack female"* is involved in the highest number of combinations (14) and 30% of all behaviors, suggesting that affiliative interactions with females indicate either social suppression and/or mating (see Supplemental Table 3). In studies examining the behavioral sequences underlying courtship, mating is more robust and involves a series of leading, quivering, and pecks at a spawning site, which was not observed here (Juntti et al., 2016). This suggests that the initial repertoire of behaviors had focal males exploring their novel ambient social environment, with subsequent spawning happening after filming in T males. Similarly, behavior central for the NTs seems to also be *"attack female"* (15 combinations), comprising 34% of relative behaviors. After the central behavior, the subsequent most common behaviors for Ts are *"pot entry"* (16%), *"pot exit"* (16%), and "*chase female*" (12%), and for NTs are *"chase female"* (16%), *"pot entry"* (12%) and "*pot exit*" (12%).

Consistent with other reports, our data shows that territorial males tend to exhibit higher reproductive success, which has been attributed in part to elevated levels of testosterone and 11-ketotestosterone compared to non-territorial counterparts(Maruska et al., 2011; Maan & Sefc, 2013; Maruska, 2015). Similarly, female preference in *A. burtoni* is largely shaped by male reproductive physiology, with secreted androgens playing an important role in female affiliation and mating bouts(Clement et al., 2005; Kidd et al., 2013). In a study done by Kidd et al., a "rapport" was established over a week before the day of spawning between dominant rank males and gravid females, suggesting that T male experience in the dyad phase may have primed reproductive success. In contrast, none of our ascending NT males showed any reproductive success (evidenced by a tally of mouthbrooding females from spawning tanks). Given that both NT and T males have similar sperm velocities (Kustan et al., 2012) and robust behaviors related to mating, it is difficult to ascertain why NT males failed to reproduce. Since hormonal changes in 11-KT in ascending males occur most robustly after 72 hours(Maruska & Fernald, 2010; Butler & Maruska, 2021), the window for reproduction would have occurred after the animals were sacrificed. Additionally, female-directed behaviors toward the focal male may have also shaped their reproductive success. For example, T and NT males both have discrete differences in their color patterns, with T males showing vibrant blue/yellow body coloration, red humeral patches, high contrasting stripes, and facial melanism (Alvarado et al., 2015b). To females, such colors are perceived by a visual system tuned to their reproductive state(Butler & Maruska, 2021), possibly affecting ascending NT reproductive success as it develops these more "attractive" color patterns. This is partially reinforced by data where females show avoidance and agonistic interactions with male androgen receptor mutants that show reduced body coloration(Howard et al., 2023).

Conclusion:

In this study, we examined the patterns and the impact of prior social status on the behavior of stably territorial and ascending male subjects. Previous research has identified distinct behavioral profiles based on male status; here, we aimed to expand on these findings by examining decision-making latency and employing transition matrices to detail sequences of behavior. Ts and ascending males employed very similar behavioral profiles in the spawning phase. Furthermore, our results indicate that while courtship behaviors occurred with equal incidence for both stably territorial and ascending males during the spawning phase, only those males that had maintained territories during the dyad phase were successful in reproducing in the subsequent spawning phase. Future research should focus on documenting and analyzing female behavioral patterns to assess whether they interact differently with males of different social pasts. Overall, the results of this study suggest that previous social experiences can shape reproductive outcomes in different environments while not affecting male-specific stereotypical social behavior.

References:

Alvarado, S. G., K. Lenkov, B. Williams, & R. D. Fernald, 2015a. Social Crowding during Development Causes Changes in GnRH1 DNA Methylation. PLOS ONE 10: e0142043.
Alvarado, S., M. Tajerian, M. Suderman, Z. Machnes, S. Pierfelice, M. Millecamps, L. S. Stone, & M. Szyf, 2015b. An epigenetic hypothesis for the genomic memory of pain. Frontiers in Cellular Neuroscience 9: 88.
Alward, B. A., A. T. Hilliard, R. A. York, & R. D. Fernald, 2019. Hormonal regulation of social ascent and temporal patterns of behavior in an African cichlid. Hormones and Behavior 107: 83–95.
Barba-Escobedo, P. A., & G. G. Gould, 2012. Visual social preferences of lone zebrafish in a novel environment: strain and anxiolytic effects. Genes, Brain and Behavior 11: 366–373.
Butler, J. M., & K. P. Maruska, 2021. Opsin Expression Varies with Reproductive State in the Cichlid Fish Astatotilapia burtoni. Integrative and Comparative Biology 61: 240–248.
Clement, T. S., K. E. Grens, & R. D. Fernald, 2005. Female affiliative preference depends on reproductive state in the African cichlid fish, Astatotilapia burtoni. Behavioral Ecology 16: 83–88.
Dengler-Crish, C. M., & K. C. Catania, 2009. Cessation of Reproduction-Related Spine Elongation After Multiple Breeding Cycles in Female Naked Mole-Rats. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology 292: 131–137.
Fang, W., D. Blakkan, G. Lee, R. Bashier, R. D. Fernald, & S. G. Alvarado, 2022. DNA methylation of the endothelin receptor B makes blue fish yellow. bioRxiv 2022.09.27.509821.
Fernald, R. D., 1977. Quantitative behavioural observations of Haplochromis burtoni under semi-natural conditions. Animal Behaviour 25: 643–653.
Fernald, R. D., 1980. Response of Male Cichlid Fish, Haplochromis burtoni, Reared in Isolation to Models of Conspecifics. Zeitschrift für Tierpsychologie 54: 85–93.
Fernald, R. D., & N. R. Hirata, 1977. Field study of Haplochromis burtoni: Quantitative behavioural observations. Animal Behaviour 25: 964–975.
Fialkowski, R., P. Aufdemberge, V. Wright, & P. Dijkstra, 2021. Radical change: temporal patterns of oxidative stress during social ascent in a dominance hierarchy. Behavioral Ecology and Sociobiology 75: 43.
Foundation, P. S., 2023. Python (Version 3.9.5) [Software]. , https://www.python.org/.
Friard, O., & M. Gamba, 2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. Methods in Ecology and Evolution 7: 1325–1330.
Gandrud, C., 2013. Reproducible Research with R and R Studio. .
Hofmann, H. A., M. E. Benson, & R. D. Fernald, 1999. Social status regulates growth rate: Consequences for life-history strategies. Proceedings of the National Academy of Sciences 96: 14171–14176.
Howard, M. R., M. G. Ramsaroop, A. P. Hoadley, L. R. Jackson, M. S. Lopez, L. A. Saenz, & B. Alward, 2023. Female cichlids attack and avoid—but will still mate with—androgen receptor mutant males that lack male-typical body coloration. bioRxiv 2023.11.02.565323.
James, G., D. Witten, T. Hastie, R. Tibshirani, & J. Taylor, 2023. An Introduction to Statistical Learning. Springer Texts in Statistics .
Juntti, S. A., A. T. Hilliard, K. R. Kent, A. Kumar, A. Nguyen, M. A. Jimenez, J. L. Loveland, P. Mourrain, & R. D. Fernald, 2016. A Neural Basis for Control of Cichlid Female Reproductive Behavior by Prostaglandin F2α. Current Biology 26: 943–949.
Khila, A., & E. Abouheif, 2008. Reproductive constraint is a developmental mechanism that maintains social harmony in advanced ant societies. Proceedings of the National Academy of Sciences 105: 17884–17889.
Kidd, M. R., L. A. O'Connell, C. E. Kidd, C. W. Chen, M. R. Fontenot, S. J. Williams, & H. A. Hofmann, 2013. Female preference for males depends on reproductive physiology in the African cichlid fish Astatotilapia burtoni. General and Comparative Endocrinology 180: 56–63.
Korzan, W. J., R. R. Robison, S. Zhao, & R. D. Fernald, 2008. Color change as a potential behavioral strategy. Hormones and behavior 54: 463–470.
Kustan, J. M., K. P. Maruska, & R. D. Fernald, 2012. Subordinate male cichlids retain reproductive competence

during social suppression. Proceedings of the Royal Society B: Biological Sciences 279: 434–443.
Laland, K. N., T. Uller, M. W. Feldman, K. Sterelny, G. B. Müller, A. Moczek, E. Jablonka, & J. Odling-Smee, 2015. The extended evolutionary synthesis: its structure, assumptions and predictions. Proceedings of the Royal Society B: Biological Sciences 282: 20151019.
Maan, M. E., & K. M. Sefc, 2013. Colour variation in cichlid fish: developmental mechanisms, selective pressures and evolutionary consequences. Seminars in cell & developmental biology 24: 516–528.
Maruska, K. P., 2015. Social Transitions Cause Rapid Behavioral and Neuroendocrine Changes. Integrative and Comparative Biology 55: 294–306.
Maruska, K. P., L. Becker, A. Neboori, & R. D. Fernald, 2013. Social descent with territory loss causes rapid behavioral, endocrine and transcriptional changes in the brain. The Journal of experimental biology 216: 3656–3666.
Maruska, K. P., & R. D. Fernald, 2010. Behavioral and physiological plasticity: rapid changes during social ascent in an African cichlid fish. Hormones and behavior 58: 230–240.
Maruska, K. P., & R. D. Fernald, 2018. Astatotilapia burtoni: A model system for analyzing the neurobiology of behavior. ACS Chemical Neuroscience 9: 1951–1962.
Maruska, K. P., B. Levavi-Sivan, J. Biran, & R. D. Fernald, 2011. Plasticity of the Reproductive Axis Caused by Social Status Change in an African Cichlid Fish: I. Pituitary Gonadotropins. Endocrinology 152: 281–290.
Nascimento, B. G. do, & C. Maximino, 2023. Social investigation and social novelty in zebrafish: Roles of salience and novelty. Behavioural Processes 210: 104903.
Seehausen, O., & D. Schluter, 2004. Malemale competition and nuptialcolour displacement as a diversifying force in Lake Victoria cichlid fishes. Proceedings of the Royal Society of London. Series B: Biological Sciences 271: 1345–1353.
Summers, C. H., G. L. Forster, W. J. Korzan, M. J. Watt, E. T. Larson, Ø. Øverli, E. Höglund, P. J. Ronan, T. R. Summers, K. J. Renner, & N. Greenberg, 2005. Dynamics and mechanics of social rank reversal. Journal of Comparative Physiology A 191: 241–252.
Thorley, J., N. Katlein, K. Goddard, M. Zöttl, & T. Clutton-Brock, 2018. Reproduction triggers adaptive increases in body size in female mole-rats. Proceedings of the Royal Society B 285: 20180897.

Figures

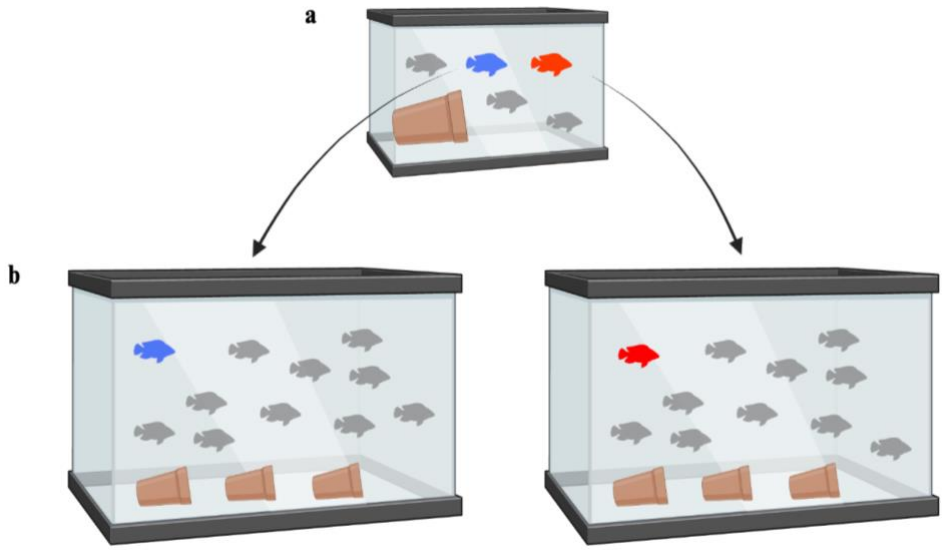

Fig. I Illustration of the behavioral assays used. a) In dyad assay, two males and three females (gray) were placed in a tank with brown gravel and half of a terracotta pot as a territory (n=4). After a month of induction, males would either ascend to T status (red) or NT status (blue) and were moved into a spawning assay. b) in spawning assay, each of the males were put in a tank with three terracotta pots, brown gravel and 10 adult females

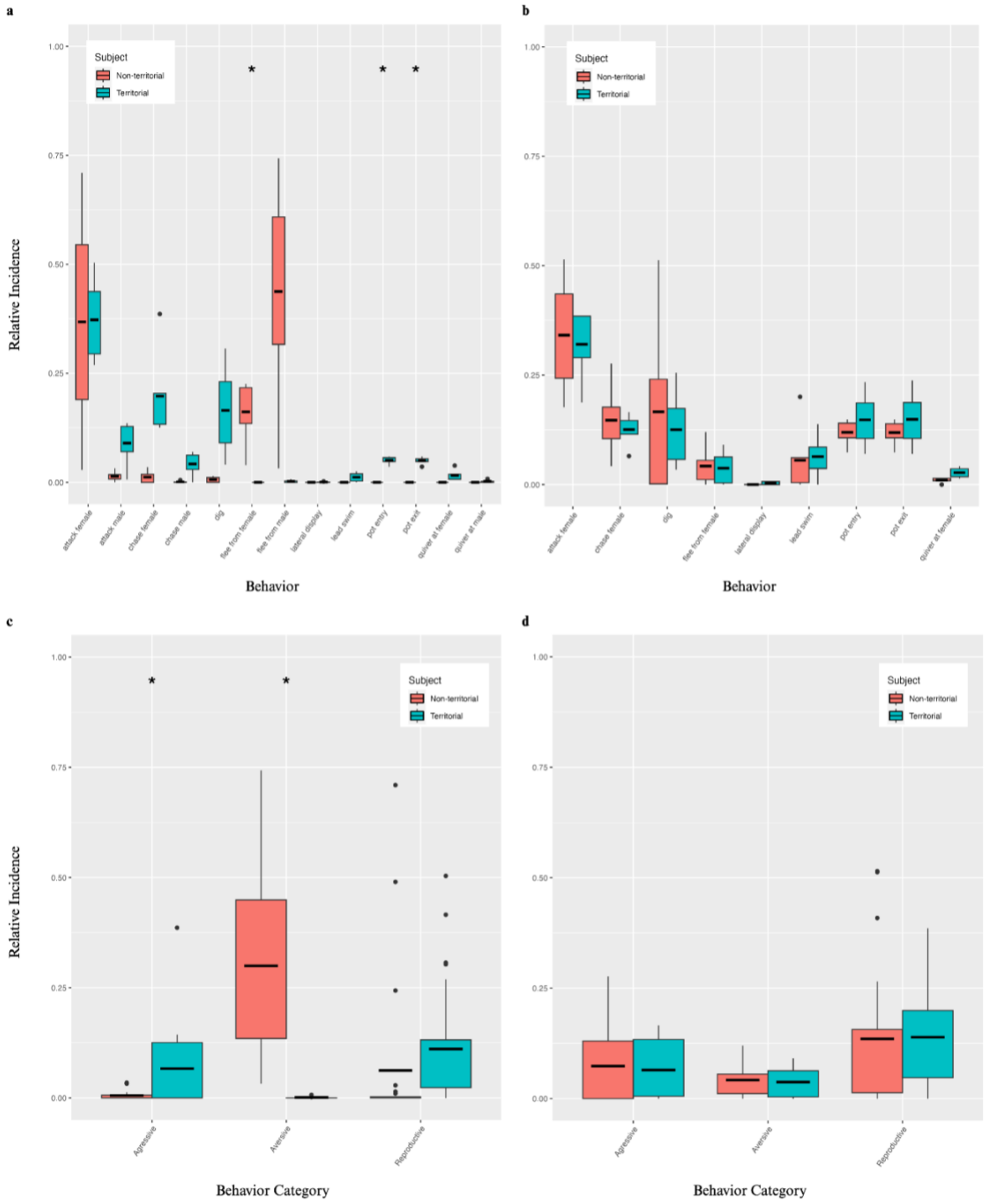

Fig. II Illustration of individual and categorical behavioral incidences of territorial and non-territorial males in dyad and spawn assays. a) We observed differences between the territorial and non-territorial males in the dyad assay in the following behaviors: *"flee from femal*e" ($p < 0.05$), *"pot entry*" ($p<0.01$), "*pot exit"* ($p<0.01$). b) There were no differences in behavior between territorial and previously non-territorial males in the spawn assay c) T males engaged in more Aggressive ($p<0.01$) and Aversive ($p<0.05$) behaviors in the dyad assay. d) Ts and previously-NTs

did not differ in the incidence of behavioral categories in spawn assay. The box plot illustrates the distribution of data as follows: the upper edge of the box represents the 75th percentile, the horizontal line inside the box marks the mean, and the lower edge of the box represents the 25th percentile. Additionally, the line extending above the box depicts the range of values from the 75th percentile to the maximum, while the line extending below the box shows the range of values from the minimum to the 25th percentile. Asterisks denote significance ($p<0.05$)

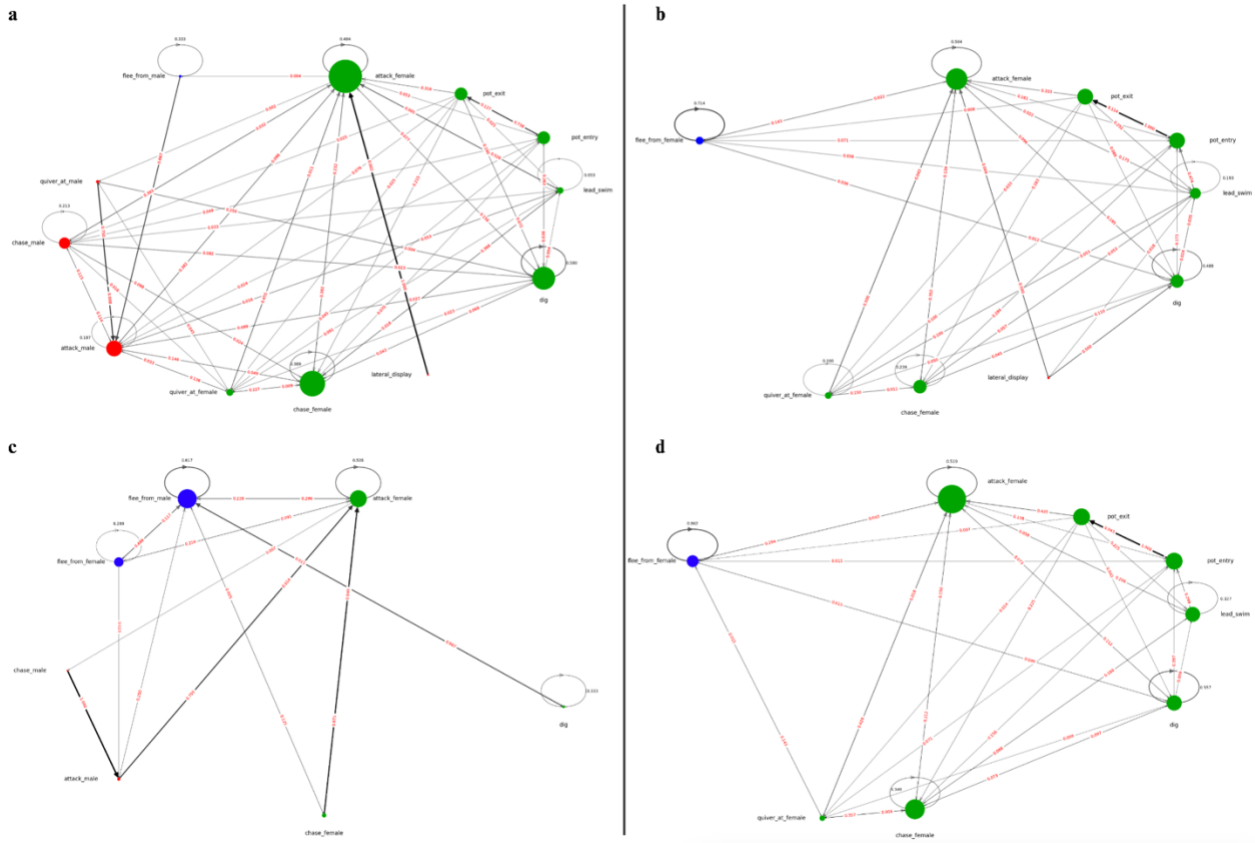

Fig. III Illustration of the Markov chain analysis conducted to examine the transition probabilities of a) Ts in dyad assay, b) Ts in spawn assay, c) NTs in dyad assay and d) NTs in spawn assay. There are 9 nodes, each representing a behavior. The color of the node corresponds to the category of the particular behavior: aggressive in red, aversive in blue and reproductive in green (for ethogram, see Supplemental Table 1). The relative sizes of the nodes reflect how frequently each behavior occurred. The thickness of the connecting lines between nodes represents how likely one behavior is to succeed another. The numbers within the plot are the probabilities of the particular behavioral transitions. The node at which the number is closest to is the start behavior and the node to which the arrow connects to is the transitioned behavior. The arrows can connect both ways, however, if the probability value is absent close to the node, it means that that behavioral transition is one-directional only. The probabilities in black represent the likelihood of the particular behavior transitioning into itself. Overall, the analysis reveals a drastic change in behavioral transitions of previously-NTs once they were allowed to ascend