Social isolation reduces metabolic rate and social preference in Wild-type Zebrafish *(Danio rerio)*

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Abstract

The adaptive and maladaptive effects of social isolation in various animal models, especially under pathogen stress, have generated particular interest since the COVID-19 pandemic. We examined metabolic, social and movement outcomes induced by a month of visual and olfactory social isolation in adult wild-type zebrafish. Our primary interest was examining metabolic savings and changes in social preference, with movement reduction as a manipulation check for effective social isolation. We found that socially isolated fish demonstrated a significant decrease in metabolic rate, social preference and average speed of movement compared to the pre-intervention baseline. The reduction in metabolic rate could lead to contextually beneficial energy expenditure savings for the fish; however, the more mixed effects of reduced social preference and average speed of movement might only be helpful in environments with a high predatory threat and high food and mate abundance. This study opens up various possibilities to explore the adaptive significance

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of social isolation in various ecological frameworks.
Keywords: social isolation, metabolic rate, conspecific social preference, average speed, movement

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1. Introduction

The adaptive and maladaptive effects of social isolation in various animal models, especially under pathogen stress, have generated particular interest since the COVID-19 pandemic. Investigations into the mechanisms underpinning the behavioural effects of social isolation require work on non-human model organisms. Significant movement reduction and other changes in movement parameters are observed as outcomes of social isolation (Becker et al., 2021), similar to what is found in different models of depression (WHO, 2003; Mathers et al., 2004; Kupferberg et al., 2016; Pan et al., 2019; Wüthrich et al., 2022).

Zebrafish, like humans, are very social creatures and demonstrate complex conspecific social interactions with the formation of social hierarchies, exhibiting behaviours like shoaling and schooling (Fontana et al., 2022). Group behaviour is posited to help escape predators, collective foraging, and mating success (Fontana et al., 2022). When social fish are experimentally isolated from their conspecifics, they exhibit consequences similar to symptoms of human depression, such as reduced locomotor activity, interest in their environment, decreased foraging, and changes in serotonergic and dopaminergic pathways associated with mood regulation (Shams et al., 2018). Social isolation has been used to induce depressive behaviour in various animal models, including rodents and zebrafish, to investigate multiple aspects of

social behaviour and stress responses (Lachowicz et al., 2021). Long-term social isolation significantly reduces social preference and locomotor activity in zebrafish, whereas brief periods have less pronounced effects on behaviour (Shams et al., 2018; Singh et al., 2023).

These reduced locomotor effects of social isolation could have adaptive roles in specific ecological contexts. The infection-defence hypothesis, for instance, posits that movement reduction can play a protective role as an evolutionary adaptive strategy (Anders et al., 2013). When the immune system responds to pathogens, it triggers inflammatory reactions that can affect the nervous system (Tohidpour et al., 2017), including reduced movement, which lowers infection by other conspecifics in the social group (Miller and Raison, 2016). However, infection can also induce other complex and seemingly contradictory mood and motor behaviour changes, making inferences difficult. For example, microbial neuraminidase administration has led to anxiety-like behaviour in the form of increased grooming behaviour as well as freezing in rodents (Dantzer et al., 2008; León-Rodríguez et al., 2022). Moreover, in Parkinson's' patients, chronic L-Dopa treatment has been shown to increase neuroinflammatory markers in the brain, particularly in the basal ganglia, involved in movement control, which can manifest as involuntary, frantic muscle movements or dyskinesia (Eusebi et al., 2018; Bishop, 2019). Alternative hypotheses propose that stress-triggered movement reduction, such as thanatosis, can provide protective advantages of reducing contact with predators (Humphreys and Ruxton, 2018; Skelhorn, 2018) or even parasites (Kralj-Fišer and Gregorič, 2019).

In an evolutionary context, social withdrawal and decreased activity lev-

els can have apparent drawbacks: these can lead to diminished exploration of new environments, leading to fewer opportunities for foraging and mating (Guo et al., 2022). The adaptive value of movement reduction due to social isolation may lie in being triggered context-dependent to effectively function as a survival strategy around movement decisions that factor in environmental cues that indicate risks versus benefits.

A mechanism through which movement reduction could benefit survival is through the decrease in metabolic costs. We may establish metabolic savings as advantageous in organisms that lead to improved survival (e.g., through lower oxidative stress) and, indirectly or directly, to improved gamete quality or quantity (Vézina et al., 2006; Palstra et al., 2010). Metabolic savings can offset the foraging opportunity costs of reduced movement (Schubert et al., 2010). Further, improved gamete quality (Kekäläinen and Evans, 2018) may also offset the disadvantage of reduced mating opportunities (Schubert et al., 2010; Kekäläinen and Evans, 2018).

Metabolic rate, correlated with body mass and temperature in phylogenetically specific ways, is commonly measured through direct and indirect calorimetry (Hardewig et al., 1991; Hackney, 2016). Direct calorimetry measures the amount of heat directly produced by the body. In contrast, indirect calorimetry quantifies oxygen utilisation and carbon dioxide production as proxy measures correlating it to tissue ATP production (Hackney, 2016; Ravelli et al., 2023). The latter is more commonly used in human beings (Hackney, 2016). Heart rate monitors and motion sensors are popular devices that measure whole-body correlates of autonomic nervous system activation and energy expenditure (Hackney, 2016; Kenny et al., 2017).

Our study attempted to quantify the metabolic savings and change in social behaviour that correlate with reduced movement due to social isolation in adult wild-type zebrafish (*Danio rerio*). We used zebrafish since they are a powerful model system with a formidable suite of genetic, developmental and functional neuroimaging tools that enable the investigation of the neural basis of behaviour (Howe et al., 2013; Marí-Beffa et al., 2021). In fish, dissolved oxygen consumption in closed water tanks is considered a viable proxy for metabolic rate, easily measured using respirometry (Cech and Brauner, 2011; Chabot et al., 2016).

Our study sought to quantify metabolic, social and locomotor changes induced by social isolation in zebrafish. We hypothesised that social isolation would reduce the metabolic rate, locomotion rate and preference for conspecific social interaction.

2. Methodology

2.1. Subjects

This quantitative experimental study uses a within-subject design that utilises adult wild-type zebrafish (*Danio rerio*) (6-12 months old). We procured all fish from a pet store in Daryaganj, New Delhi, India, sourced from local water bodies. A 12:12::light:dark circadian cycle (from 10 am to 10 pm), at pH of 7.50–8.50, temperature of approximately 28°C - 30°C, and conductivity of 650 µS/cm - 700 µS/cm at at Ashoka University, Sonipat, Haryana, India, was maintained. We fed the fishes ad libitum Tetra-Tetramin flakes once daily and conducted experiments between 10 am and 4 pm (Singh et al., 2023).

We randomly picked out 30 fish from the procured population. However, complete data collection for analysis comprised 26 fish (12 males and 14 females). We individually housed all fish in a large glass tank separated by transparent barriers for easy identification. The transparent barriers and shared water ensured that visual and olfactory cues were maintained. Four fish were randomly chosen from the same population on the day of the experiment for the display shoal used in the social preference assay.

2.2. Social Isolation

We isolated experimental fish in plastic containers covered with a minimally translucent membrane that allowed for only the passage of ambient light, inhibiting conspecific visual cues. Further, we only used freshly prepared embryo water for social isolation, allowing us to inhibit olfactory cues. We prepared a stock solution of embryo water to ensure consistency across experiments, which we diluted with fresh distilled water to prepare the isolation medium for each experimental fish. The embryo water stock solution was prepared using the guidance of the Cold Spring Harbor Laboratory (2011) (Williams and Renquist, 2016) and stored in a climate-controlled environment. 16.5 mL of this stock solution was dissolved in 1 litre of distilled water to prepare the embryo water solution separately for each isolated fish. We isolated each fish for 1 month (30 days) before testing.

2.3. Operationalisation and Experimental Design

2.3.1. Metabolic Rate

We operationalised metabolic rate as the amount of dissolved oxygen consumed by each experimental fish in a given time frame. We determined the

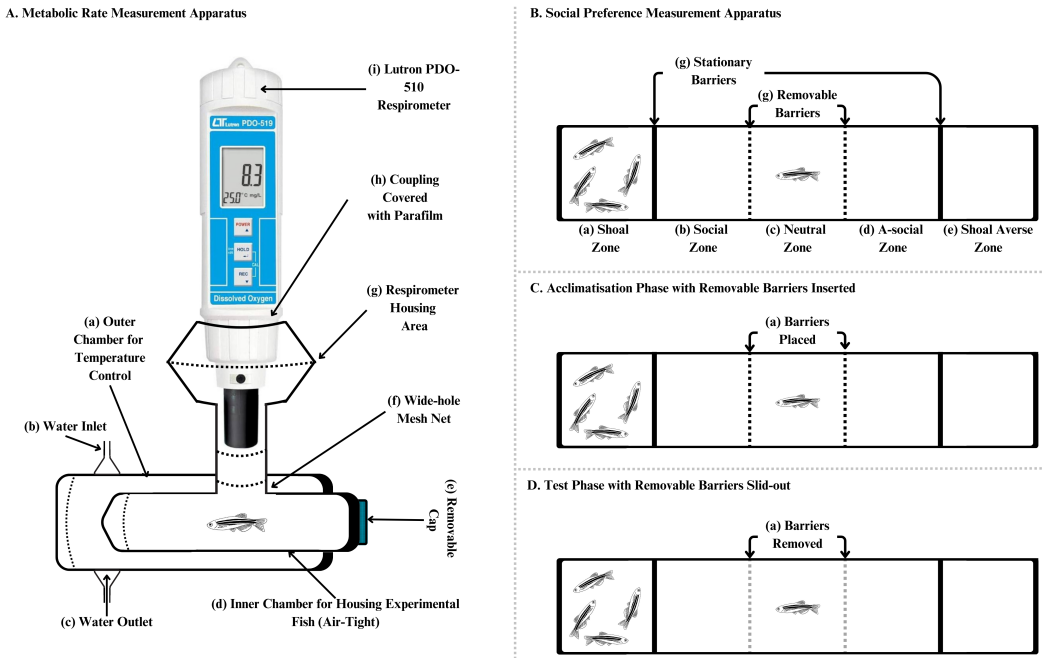

Figure 1: (A) Metabolic Rate Measurement Apparatus, (B) Diagrammatic representation of the social interaction recording setup (Ogi et al., 2021), (C) Acclimatisation phase where the experimental fish remains in the neutral zone for 30 seconds, (D) Test phase in which we raised the removable barriers and the experimental fish was allowed to make its preference for 300 seconds or 5 minutes.

amount of oxygen the experimental fish consumed by measuring the dissolved oxygen present over time in the experimental setup using a respirometer (Cech and Brauner, 2011). Our experimental setup, built in-house, consisted of a large external chamber connected to a temperature control system (a) in Figure 1(A). An inlet and outlet channel helped circulate water in the outer chamber to control for temperature, (b) and (c), respectively, in Figure 1(A). Nested inside was a smaller inner chamber with approximately 100 mL (d) capacity in Figure 1(A). This inner chamber served as the test chamber for

the experimental fish. We first inserted an experimental fish into the inner chamber from the upper respirometer housing slot (g) in Figure 1(A). Next, a wide-holed net was inserted into the same slot to prevent the experimental fish from swimming upward (f) in Figure 1(A). Subsequently, the respirometer, a Lutron PDO-519 dissolved oxygen metre, was mounted into the designed housing (i) in Figure 1(A), sealing the coupling using parafilm to ensure the inner chamber is airtight (h) in Figure 1(A). There was no water exchange between the two chambers, and the inner chamber was airtight, ensuring accurate dissolved oxygen measurements. The difference in dissolved oxygen presence over time was a direct indication of oxygen consumption by the experimental fish and, therefore, was used to calculate a measure of its metabolic rate (Voutilainen et al., 2011; Francis-Floyd, 2020; Divakaruni and Jastroch, 2022).

Once we placed the parafilm to seal the inner chamber, we began recording readings as reflected by the respirometer. We took one reading every minute for a total of 90 minutes. During our initial trials, we experimented with different recording durations and found that after 90 minutes, the amount of dissolved oxygen in the chamber reached dangerously low levels. We, therefore, limited our recording time to 90 minutes at maximum.

2.3.2. Social Preference

We considered social preference as the bias to live near and interact with conspecifics and the motivation to identify and approach them (Liu et al., 2016). We measured social preference in terms of the amount of time spent by the experimental fish socialising with its conspecifics vis-à-vis the time spent away from them. We used a uniformly lit, $50 \times 10 \times 12cm^3$ plexiglass

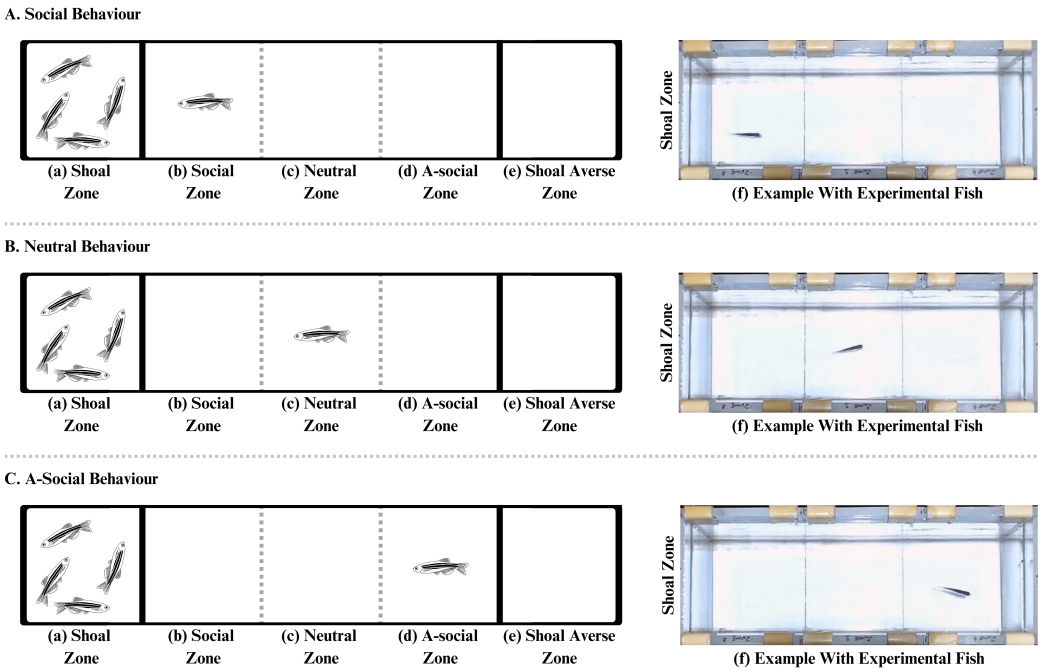

Figure 2: (A) Experimental fish demonstrating shoal preference by spending a majority of the time of the test phase in the shoal zone, (B) Experimental fish demonstrating neutral behaviour or indecisiveness by spending a majority of the time of the test phase in the neutral zone, (C) Experimental fish demonstrating a-social or shoal averse behaviour by spending a majority of the time of the test phase in the a-social zone

tank divided into five equal chambers laterally, as shown in Figure 1(B). We utilised an opaque black membrane to cover all four sides of the tank to prevent external confounding stimuli. The tank consisted of four barriers, two stationary and two removable, as shown in Figure 1(B) (Pham et al., 2012). The shoal, composed of 4 display adult zebrafish, was placed at a terminal zone, (a) in Figure 1(B), while the opposite end, (e) in Figure 1(B), was left empty. In the acclimatisation phase, the experimental fish was placed in the neutral zone (c) in Figure 1(B) and was allowed to acclimate for 30

seconds (Pham et al., 2012). Following this, in the test phase, we took out the removable barriers (g) in Figure 1(B) and allowed the experimental fish to explore the tank for 300 seconds (Pham et al., 2012). We used a Lenovo 300 FHD 1080P Megapixel webcam (Lenovo, 2020) to record all trials in .MP4 format. We mounted the camera on a tripod 60 cm from the bottom of the tank to capture a complete top view. An experimental fish was categorised as 'social' if it spent a majority of the test phase in the zone towards the shoal or the 'social' zone, (b) in Figure 2(A) and 'a-social' if it spends a majority of the test phase in the zone away from the shoal or the 'a-social' zone, (d) in Figure 2(C). To account for lateral bias and its confounding effects, two trials were conducted for each experimental fish, alternating the position of the shoal (a) and (e) in Figure 1(B) (Pham et al., 2012).

2.3.3. Average Speed of Movement

We used the positional information obtained in the social preference identification to extract further the distance covered by each experimental fish in the test phase. We estimatd the experimental fish's average speed throughout the trials using this information, looking at the change in position over time.

2.3.4. YOLO Based Fish Tracking

We converted each experimental fish video into greyscale and cropped it to only include the three central zones, (b), (c), and (d) in Figure 2(A). Subsequently, the videos were stored as .MP4 files and cropped for any extra recording time to a fixed duration of 5 minutes, starting from the moment the removable barriers are raised, (g) in Figure 1(B). Figure 3 shows an example

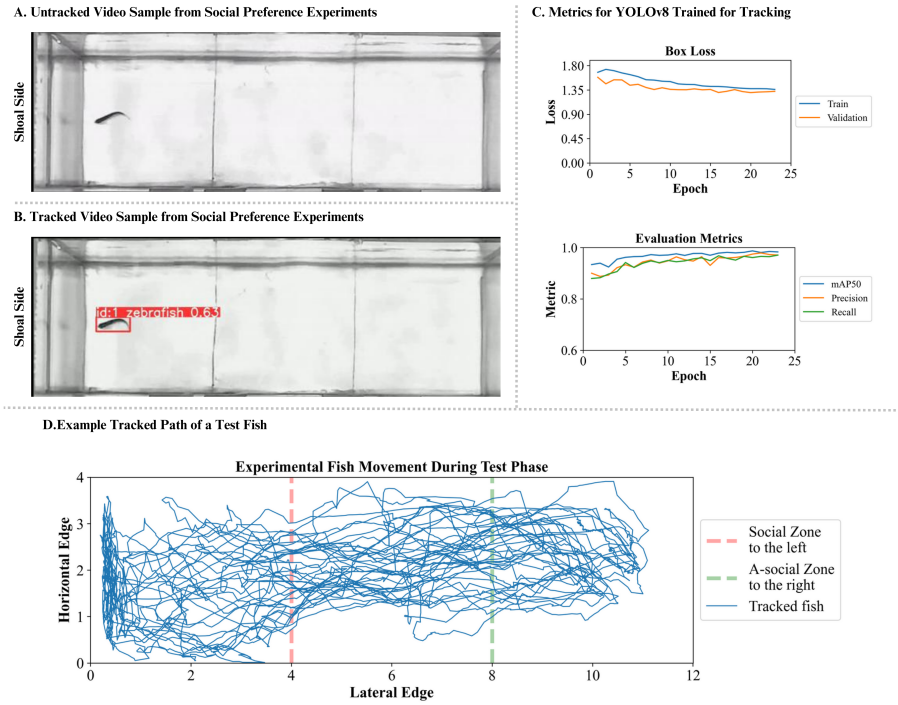

Figure 3: (A) Raw videos from the social preference experiments after corrections (cropping, grayscale conversion, and conversion to MP4), (B) Object tracking carried out on the corrected video from the social preference experiments, (C) The loss and evaluation metric curves for the trained model, (D) Scatter plot depicting the path of the tracked fish.

of a single frame from a corrected video. Subsequently, we used YOLOv8 and Python to carry out object tracking and analysis for the corrected videos.

To train YOLOv8, approximately 100 frames from each recorded video from the test phase were sampled. The sampling process yielded close to 8000 frames, annotated using Roboflow (Dwyer et al., 2024). These annotated frames were then split into 70%, 20%, and 10%, for train, test, and validation, respectively, and YOLOv8 was trained to identify and localise

the experimental fish (Jocher et al., 2023; Varghese and M., 2024; Bichri et al., 2024). The metric we chose to evaluate our model was mean average precision (mAP), where we thresholded at an intersection over union (IoU) of 0.5 to optimally tradeoff between train time and performance (Henderson and Ferrari, 2017). Further, we trained for 23 epochs to ensure that model overfitting did not occur. Our model was able to achieve an mAP of 98.5. We used this model to identify the position of the experimental fish at each frame and used this information in our downstream analysis. The positional information, once received, was used to estimate the amount of time spent by the experimental fish in the social and a-social zones.

2.4. Protocol

On day 0, we first recorded the social preference alongside the movement rate of the fish. Subsequently, following a 15-minute rest period, the metabolic rate of the experimental fish was recorded. The experimental fish was placed in social isolation immediately following the cessation of the metabolic rate recording. We ensured that animal transfer occurred carefully using sterilised fresh nets to minimise the transfer of conspecific olfactory cues and minimise transfer-induced stress. We then kept the experimental fish in their isolation chamber for 30 days. On the 31st day, we recorded the social preference and metabolic rate in the same manner as on day 0.

2.5. Analysis

2.5.1. Metabolic Rate

Our data for metabolic rate consisted of 90 individual time-series points for 26 fish at two stages, i.e., before and after social isolation. Based on

data normality, checked using a Shapiro-Wilk test, we conducted a paired sample t-test for normal data or a Mann-Whitney U-test for non-normal data. We checked for differences in the data measured before and after social isolation at each time point after we placed the fish in the inner chamber of the experimental setup.

2.5.2. Social Preference and Average Speed of Movement

We compared the amount of time spent before and after social isolation in the social and a-social zones, as well as the average speed of movement before and after social isolation in the test phase, using paired sample t-tests for normal data and a Mann-Whitney U-test for non-normal data, after checking for data normality using a Shapiro-Wilk test.

3. Results

The rate of oxygen consumption after the administration of a month-long period of social isolation was significantly lower than the pre-intervention stage at the population level Figure 4(A) (Mann–Whitney U test, $p \leq 0.05$, $d = -0.550$, $N = 26$: 12 male fish and 14 female fish). This decrease in the oxygen consumption rate indicated a lower metabolic rate post-intervention (Appendix A).

We also checked for metabolic rate measurement replicability by repeated measurements on consecutive days on a separate control batch of fish. The rate at which fish consumed oxygen in water trials on the two consecutive days was replicable. There was no significant difference observed between oxygen consumption rate on both days for the overall population Figure 4(B)

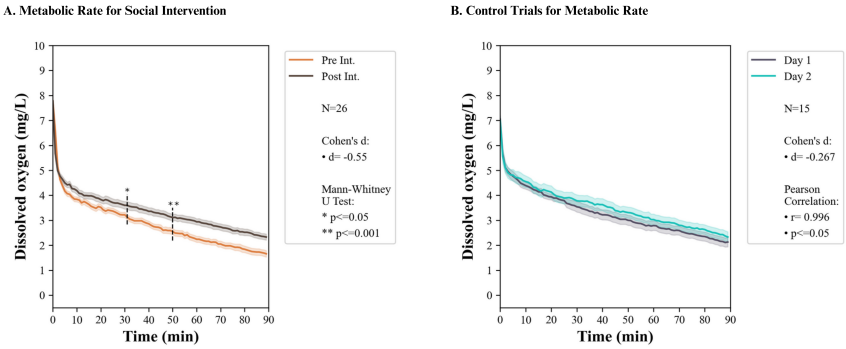

Figure 4: (A) Higher dissolved oxygen level in the metabolic rate recording set-up after the administration of social isolation, indicating a lower metabolic rate. There was a statistically significant ($p \leq 0.05$)) difference between the oxygen consumption pre- and post-intervention, (B) No significant difference at any time point between the metabolic rate recordings carried out on consecutive days (Pearson's correlation coefficient: 0.996, $p \leq 0.05$).

(Mann–Whitney U test, $p > 0.05$, $d = -0.267$, $N = 15$: 9 male fish and 6 female fish)(Appendix G). Batch identity influenced the baseline measure, but repeated measures showed a significantly high correlation of measurements across days of measurement for a given batch of fish. (Pearson's correlation, r= 0.996, $p \leq 0.05$, $d = -0.267$, $N = 15$: 9 male and 6 female fish).

The one-month isolation treatment also significantly reduced the average speed at which the fish travelled post-intervention ($M = 2.22$, $SD = 0.425$) compared to the pre-intervention stage ($M = 2.520$, $SD = 0.472$) at the population level Figure 5(A) (Paired sample t-test, $p \leq 0.05$, $d = 0.677$, $N = 26$: 12 male fish and 14 female fish) (Appendix B).

The social preference, i.e., the proportion of time spent by the fish in the social zone, significantly decreased after social isolation ($M = 0.664$, $SD = 0.179$) when compared to the pre-intervention stage ($M = 0.843$,

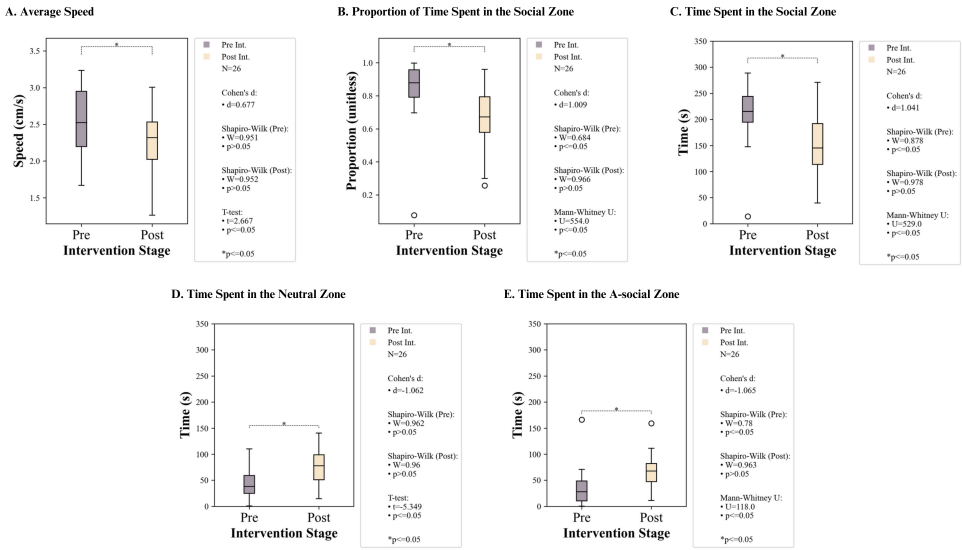

Figure 5: (A) The average speed of movement (cm/s) of the fish significantly ($p \leq 0.05$) reduced after social isolation, (B) The proportion of time the fish spent in the social zone was significantly less after social isolation, compared to the pre-intervention stage, (C) There was a statistically significant decrease ($p \leq 0.05$) in time spent by the fish in the social zone post-intervention, (D) Statistically significant rise ($p \leq 0.05$) in the time spent by the fish in the neutral (E) and a-social zones (F).

$SD = 0.183$), at the population level (Mann-Whitney U test, $p \leq 0.05$, $d = 1.009$, $N = 26$: 12 male fish and 14 female fish) Figure 5(B) (Appendix C). This period of social isolation also significantly reduced the absolute time spent by the fish in the social zone post-intervention ($M = 152.17$, $SD = 61.76$) when compared to the pre-intervention stage ($M = 213.52$, $SD = 58.37$) for the overall population Figure 5(C) (Mann–Whitney U test, $p \leq 0.05$, $d = 1.041$, $N = 26$: 12 male fish and 14 female fish)(Appendix D).

Conversely, there was a significant increase in the time spent by the fish in the a-social zone post-intervention ($M = 68.97$, $SD = 31.86$) when compared

to the pre-intervention stage ($M = 34.33$, $SD = 34.41$) at a population level (Mann–Whitney U test, $p \leq 0.05$, $d = -1.065$, $N = 26$: 12 male fish and 14 female fish); as well as in the neutral zone post-intervention ($M = 74.90$, $SD = 35.81$) when compared to pre-intervention ($M = 41.59$, $SD = 27.58$), for the overall population Figure 5 (D) and (E) (Paired sample t-test, $p \leq 0.05$, $d = -1.062$, $N = 26$: 12 male fish and 14 female fish) (Appendix E and Appendix F). Our results indicated a reduction in overall social interaction or a decrease in the preference of the experimental fish to interact with conspecifics after 1 month of social isolation.

4. Discussion

Our study examined metabolic savings and social preference in wild-type zebrafish to investigate the contextual advantages of social isolation-induced movement reduction. We contribute to the existing literature by quantifying metabolic savings accompanying behavioural changes in movement rate and social preference induced by social isolation. After one month of social isolation, we report reduced metabolic and movement rates and reduced social preference. This approach provides insights into understanding the bioenergetic state of an organism when subjected to social isolation. This aspect has been relatively understudied in prior literature that predominantly emphasises behavioural parameters.

Since social interaction and locomotion are both energy-consuming processes (Ohlberger et al., 2005; Mushtaq et al., 2014; art, 2020), the observed reduction in movement, social interaction, and metabolic rate in socially isolated fish effectively demonstrates a potentially advantageous decrease in

energy expenditure. Past literature has looked at some of these variables separately but found results that corroborate ours: varied activity levels between fish correlate with differences in energy expenditure (Brownscombe et al., 2017), and the energetic costs of swimming (Ohlberger et al., 2005) are higher than the energetic costs of simply being still (Speers-Roesch et al., 2018), supporting our overall finding that lowered movement lowers energy expenditure (Ohlberger et al., 2005; Gerlai et al., 2009; Millidine et al., 2009; Lachowicz et al., 2021).

However, reduced locomotion could also lead to a reduction in foraging behaviour and, therefore, reduced energy intake in resource-constrained ecological contexts (Weimerskirch et al., 2003; Åke Norberg, 2021; Li et al., 2022). Further, socially withdrawn fish have been found to compete less for resources compared to fish swimming in shoals (Åke Norberg, 2021). While one can implicate reduced foraging in reduced energy expenditure, it also involves the reduced consumption of food resources (Weimerskirch et al., 2003). Therefore, one would only expect energetic savings for socially withdrawn fish in environments with ample availability of resources and less competition required for food. These socially withdrawn fish could repurpose these metabolic savings for various bodily functions, such as reproductive or immune investment. In resource-rich environments, one could further investigate whether these energetic savings we observed lead to increased reproductive fitness. Likewise, future studies could examine whether this increased energetic saving in resource-rich environments could also lead to a strengthened bodily immune response, which could, in turn, reduce the chances of infections caused by parasites and pathogens, thereby increasing

chances of survival (Roth et al., 2010; Bird, 2019; Zhang and Lauder, 2023). Immune system aside, fish showing a reduced average speed of movement are more protected from the transmission of pathogens and parasites when compared to fish that show increased shoaling behaviour (Li et al., 2022). These fish are less likely to come into contact with other fish, pathogens and parasites, reducing the overall infection transmission rate (Li et al., 2022). Therefore, lower locomotion and social interaction could improve immunity, both through reduced contact with sources of pathogens and through the investment of energetic savings in immune responses to pathogens.

Socially withdrawn fish showing reduced activity levels also encounter fewer predators compared to fish swimming in shoals, reducing their chances of getting preyed upon (Ioannou and Krause, 2008). This reduction is noteworthy in environments with high predatory threats. Hence, a reduction in infection transmission rate and decreased predatory exposure could increase the chance of survival, which could also indirectly lead to an increase in their reproductive fitness — investigation in future experiments is required to verify this.

The primary factor to assess reproductive fitness is the reproduction rate. We aim to collect standardised data to determine the reproduction rate after social isolation and gather replicable data to facilitate definitive conclusions. Additionally, we plan to substantiate the contextual advantages of altered metabolic rate, social preference, and movement within specific ecological frameworks by simulating contexts characterised by high food resource availability and increased predator presence.

Zebrafish show behavioural changes similar to those seen in humans after

being socially isolated for a specific period (Lachowicz et al., 2021; de Abreu et al., 2022). We quantified these behaviours in our study and confirmed earlier literature on the effects of social isolation. Socially isolated zebrafish show a decrease in the overall rate of movement and foraging behaviour, reduced interest in exploring the environment, and poor preference or motivation for social interaction (Shams et al., 2017, 2018; Tunbak et al., 2020).

Fish that were raised in complete isolation since birth, when tested in adulthood, demonstrated significantly less social preference when compared to socially raised fish (Tunbak et al., 2020). Social isolation did not drive social interaction but increased social aversion. The period of isolation matters: adult zebrafish isolated acutely for 24 hours did not show changes in their locomotor activity and social preference when exposed to their conspecifics (Shams et al., 2018), but those isolated for a prolonged period of 6 months showed significant changes in locomotor activity and reduced social preference (Shams et al., 2018). Thus, we inferred that increased social isolation leads to decreased social preference and is associated with changes in locomotor activity. Owing to logistical constraints, we chose 1 month (30 days) as our duration of isolation. Our findings are not only replicated - we also add data for an intermediate period of isolation between 24 hours and 6 months, where we find that the effects of social isolation for a month are similar to the changes seen after 6 months.

The changes in metabolic rate and movement parameters observed after one month of social isolation are unlikely due to ageing, as age-related physiological changes in zebrafish typically manifest around two years of age (Kishi et al., 2003; KHOR et al., 2019). Additionally, between 6 months and

22 months, zebrafish demonstrate stable physiological and metabolic activity, with no significant changes in body mass index, body length, and oxygen consumption, further indicating that the observed changes are an effect of the intervention of social isolation (Yang et al., 2019).

We must address a few limitations of our study. The small space of the inner chamber in the metabolic rate measurement setup could have acted as a stressor for the fish. Further, keeping the fish in an airtight chamber for 90 minutes could also have induced hypoxia, another potential stressor. These stressors could have impacted the metabolic rate measurements taken in this study. However, the high repeatability of the metabolic rates we measured over consecutive days suggests that the fish's metabolic rates remained consistent over 24 hours.

This study did not assess anxiety-like behaviours, potentially induced after social isolation; however, another parallel study in our laboratory did, explicitly looking at anxious behaviours, such as thigmotaxis (wall-hugging locomotion) and irregular zig-zag movements, to understand if anxiety-like behaviours were also produced alongside depression-like behaviours after social isolation (Lachowicz et al., 2021).

To conclude, this study quantified metabolic activity, social interactions, and movement patterns to identify parameters relevant to understanding the potential advantages of social withdrawal induced by social isolation in wild-type zebrafish in specific contexts. By addressing a significant gap in existing literature, this research sheds light on the adaptive significance of traits associated with depressive behaviour, such as reduced movement rate, social preference, and metabolic rate — linked to reduced energy expenditure

within relevant contexts. Reduced energy expenditure has several context-specific advantages that could improve the chances of survival by increasing reproductive fitness. Lastly, it is essential to note that this study does not aim to delve into the adaptive value of depressive disorder but instead focuses on understanding the adaptive significance of its constituent traits. This study opens many possibilities to explore depressive behaviours within a wide range of simulations that accurately mimic specific ecological frameworks to examine the adaptive significance of these traits.

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Author contributions statement

AG and BKR designed the study; AG and AMR made the apparatus; AG, AMR, PM, SR, KB, SS, MP, and TT collected the data; AG and AMR performed data analysis; and AG, BKR, AMR, PM, SR wrote the manuscript.

Competing Interest

The authors declare no competing interest.

Additional Information

We followed CSPCA national and institutional guidelines for the care and use of animals. Ethical approval was obtained from Ashoka University's Institutional Animal Ethics Committee (Approval no. ASHOKA/IAEC/2/2022/6).

Data Availability

The code is available at the GitHub Repository here :`https://github.com/mraoaakash/EcoAdvDep`, and the data is available at the Zenodo repository here `https://doi.org/10.5281/zenodo.13755105`

Appendix A. Metabolic Rate

	Between Pre and Post SI
Cohen's D	-0.55

Table A.1: Detailed statistics for metabolic rate before and after social isolation (SI).

Appendix B. Average Speed of Movement

Test	**Statistic**	**Between Pre and Post SI**	**Pre-SI**	**Post-SI**
Shapiro-Wilk	W	-	0.951	0.952
Shapiro-Wilk	p	-	0.240	0.255
Mean	-	-	2.520	2.222
Standard Deviation	-	-	0.472	0.426
Cohen's d	d	0.677	-	-
Paired t-test	t	2.666	-	-
Paired t-test	p	0.013	-	-

Table B.2: Detailed statistics for average speed of movement before and after social isolation (SI).

Appendix C. Proportion of Time Spent in Social Zone

Test	**Statistic**	**Between Pre and Post SI**	**Pre-SI**	**Post-SI**
Shapiro-Wilk	W	-	0.684	0.966
Shapiro-Wilk	p	-	3.241×10^{-6}	0.532
Mean	-	-	0.843	0.663
Standard Deviation	-	-	0.183	0.18
Cohen's d	d	1.009	-	-
Mann-Whitney U-test	U	554	-	-
Mann-Whitney U-test	p	8.016×10^{-5}	-	-

Table C.3: Detailed statistics for proportion of time spent in social zone before and after social isolation (SI).

Appendix D. Time Spent in the Social Zone

Test	**Statistic**	**Between Pre and Post SI**	**Pre-SI**	**Post-SI**
Shapiro-Wilk	W	-	0.878	0.978
Shapiro-Wilk	p	-	0.005	0.821
Mean	-	-	213.522	152.176
Standard Deviation	-	-	58.378	61.767
Cohen's d	d	1.041	-	-
Mann-Whitney U-test	U	529	-	-
Mann-Whitney U-test	p	4.896×10^{-4}	-	-

Table D.4: Detailed statistics for time spent in social zone before and after social isolation (SI).

Appendix E. Time Spent in the Neutral Zone

Test	**Statistic**	**Between Pre and Post SI**	**Pre-SI**	**Post-SI**
Shapiro-Wilk	W	-	0.962	0.960
Shapiro-Wilk	p	-	0.43	0.399
Mean	-	-	41.598	74.901
Standard Deviation	-	-	27.581	35.82
Cohen's d	d	-1.062	-	-
Paired t-test	t	-5.349	-	-
Paired t-test	p	1.515×10^{-5}	-	-

Table E.5: Detailed statistics for time spent in neutral zone before and after social isolation (SI).

Appendix F. Time spent in the A-Social Zone

Test	**Statistic**	**Between Pre and Post SI**	**Pre-SI**	**Post-SI**
Shapiro-Wilk	W	-	0.78	0.963
Shapiro-Wilk	p	-	8.141×10^{-5}	0.46
Mean	-	-	34.332	68.97
Standard Deviation	-	-	34.414	31.863
Cohen's d	d	-1.065	-	-
Mann-Whitney U-test	U	118	-	-
Mann-Whitney U-test	p	5.892×10^{-5}	-	-

Table F.6: Detailed statistics for time spent in a-social zone before and after social isolation (SI).

Appendix G. Water Control Trials

	Statistic	**Between Day 1 and Day 2**
Cohen's D	D	-0.0267
Pearson's Correlation	r	0.996
Pearson's Correlation	p	1.647×10^{-95}

Table G.7: Detailed statistics for the water control trials for metabolic rate taken on consecutive days.

Appendix H. Social Isolation Intervention Protocol

Appendix H.1. Pre-Intervention

Task	Method
Social Interraction	***1.*** Introduce fish into neutral zone with barriers in place. ***2.*** Allow 30 seconds for acclimatization in neutral zone. ***3.*** Raise barriers and allow for free-swimming and exploration for 5 minutes. ***4.*** Conduct second trial alternating shoal position
Metabolic Rate	***1.*** Ensure the tank is filled with water up to its brim. ***2.*** Put the Zebrafish in the inner tank. ***3.*** Dip the respirometer in the inner tank. ***4.*** Add parafilm to ensure that the enclosure is airtight. ***5.*** Note down respirometer readings every minute. ***6.*** Take readings for 90 minutes.
Feeding	Feed up to 2 crushed pellets after both trials and metabolic rate measurements are complete.

Table H.9: Detailed Social Isolation (SI) intervention protocol at the pre-intervention stage.

Appendix H.2. Post-Intervention

Task	Method
Social Isolation Intervention	***1.*** Prepare chamber using minimally translucent container. ***2.*** Fill the chamber with freshly prepared embryo water. ***3.*** Place the experimental fish in the isolation chamber. ***4.*** Place the isolation chamber in an undisturbed location.
Isolation Phase	Conspecific visual and olfactory isolation for a period of 30 days
Social Interraction	Carry out Social Interraction trials as mentioned in the pre-intervention protocol
Metabolic Rate	Carry out metabolic rate trials as mentioned in the pre-intervention protocol
Feeding	Feed up to 2 crushed pellets each day at 10:00 am. No feeding for 24 hours prior to trials. Final feeding after both trials and metabolic rate measurements are complete.

Table H.11: Detailed Social Isolation (SI) intervention protocol at the post-intervention stage.

Appendix I. Embryo Water Solution Preparation

Social isolation was carried out in embryo water to remove olfactory cues present in the water. The embryo water was prepared using the following protocol for 60x stock developed by the Cold Spring Harbor Laboratory (2011) for the use of zebrafish embryos (Williams and Renquist, 2016):

1. In a clean glass bottle, add 2 litres of distilled water.
2. To the water, add the following salts:
   (a) $34.8g$ of Sodium Chloride ($NaCl$)
   (b) $1.6g$ of Potassium Chloride (KCl)
   (c) $5.8g$ of Calcium Chloride ($CaCl_2 2H_2O$)
   (d) $9.78g$ of Magnesium Chloride ($MgCl_2 6H_2O$.)
3. Use Sodium Hydroxide ($NaOH$) and Hydrochloric Acid (HCL) to achieve a pH of 7.2
4. 60X stock solution is now prepared.
5. To prepare a 1X solution do the following:
   (a) In a clean glass bottle, add 1 litre of distilled water.
   (b) To this water, mix $16.5mL$ of the prepared stock
   (c) To this solution, add $100\mu L$ 1% methylene blue.
   (d) 1X solution is now ready.

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