Social isolation reduces oxygen consumption, speed of movement 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 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. We found that socially isolated fish demonstrated a significant decrease in oxygen consumption, social preference and average speed of movement compared to the pre-isolation baseline. This study opens up various possibilities for refining future zebrafish experiments, and other isolated animals may allow for translating this framework to the consequences of pandemic-related social isolation in humans.

Keywords: oxygen consumption, conspecific social preference, average speed, movement

Introduction

Since the COVID-19 pandemic, the behavioural consequences of social isolation in various animal models have generated particular interest. The impact of social isolation on behaviour and its association with health-related parameters requires further investigation. Social isolation induces depressive behaviour in various animal models, including rodents [1] and zebrafish [2][3]. Our study examined the behavioural and metabolic outcomes of a month of social isolation in zebrafish.

Zebrafish, like humans, are highly social creatures that engage in complex interactions with their species [4]. They form social hierarchies and exhibit behaviours such as shoaling and schooling [4]. Group behaviour is posited to help escape predators, collective foraging, and mating success [5][6][4]. When zebrafish are experimentally isolated from their conspecifics, they exhibit consequences such as reduced locomotor activity [7][8][9], reduced interest in their environment, decreased foraging, and changes in serotonergic and dopaminergic pathways associated with mood regulation [10], similar to findings in various rodent models of depression. Long-term social isolation significantly reduces social preference and locomotor activity in zebrafish, whereas brief periods have less pronounced effects on behaviour [10][11].

Periods of social isolation are an ecologically relevant paradigm for zebrafish, which live in rain-fed streams that can dry up to form shrinking ponds, which can later be reconnected by heavy rains [12][13]. In an evolutionary context, social withdrawal and decreased activity levels

can have apparent drawbacks, as they can lead to diminished exploration of new environments, resulting in fewer opportunities for foraging and mating, as observed in Atlantic salmon [14]. However, the reduced social and locomotor activity effects of social isolation could have adaptive roles in specific ecological contexts.

An essential mechanism through which social isolation and reduced movement can affect survival is their impact on metabolic rate. The stress of social isolation [15] could either increase metabolic rates as observed in mice and fish [16][17][18], trading off against the possible metabolic savings due to reduced movement [19]. Metabolic savings have been shown to lead to improved survival by reducing ageing through lowering oxidative stress [20] or by being repurposed towards improved immune systems in mice [21] or reproductive systems in fish [22] [23]. The direction of downstream effects on reproductive investment change is also unclear - reduced swimming triggers gamete development in rainbow trout [22] and the European silver eel [23]. In contrast, reduced mating opportunities lead to sperm ageing and reduced sperm quality in zebrafish [24] or reduced sperm quantity in guppies [25].

The relationship between isolation and immunity is also unclear. The infection-defence hypothesis posits that immune reactions to pathogens can lead to reduced movement, as seen in bighorn sheep, which can play a protective role by reducing the spread of infection from other conspecifics in a social group [26]. In coral reef fish, on the other hand, the stress of infection can directly debilitate the immune system [27] [78]. In addition, infection can also induce complex and seemingly contradictory changes in mood and motor behaviour. For example, microbial neuraminidase administration led to increased grooming behaviour-related movement and bouts of freezing behaviour in rodents [28][29], making inferences difficult. Social isolation would also lead to reduced movement and is expected to reduce the spread of infection. At the same time, the concomitant reduction in foraging opportunities would lead to increased stress and immunosuppression in zebrafish [30]. Therefore, the metabolic, immune, and reproductive consequences of reduced movement due to social isolation will likely be highly context-specific and warrant further investigation. We begin by looking at the metabolic correlates of movement reduction induced by social isolation.

Metabolic rate, correlated with body mass and temperature in phylogenetically specific ways, is commonly measured through direct and indirect calorimetry [31][32]. Direct calorimetry measures the amount of heat directly produced by the body [31][33]. In contrast, indirect calorimetry quantifies oxygen utilisation and carbon dioxide production as proxy measures correlating to tissue adenosine triphosphate (ATP) production [31][33]. The latter is more commonly used in human beings [34]. Heart rate monitors and motion sensors are popular devices that measure whole-body correlates of autonomic nervous system activation and energy expenditure [31][35].

Our study aimed to quantify the metabolic, social, and locomotor changes induced by social isolation in adult wild-type zebrafish (*Danio rerio*), a powerful model system with a range of genetic, developmental, and functional neuroimaging tools for investigating the neural basis of behaviour [36][37]. We hypothesised that social isolation would reduce locomotion and, therefore, metabolic rate, and decrease the zebrafish's preference for conspecific social interaction. To measure metabolic rate, we used respirometry to assess dissolved oxygen consumption in closed water tanks, a well-established proxy for metabolic activity in fish [38][39].

Methodology

Subjects

This quantitative experimental study employs a within-subject design using 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. The fish were housed collectively during acclimation to the lab for two weeks before the experiment at in a ZebTec Active Blue - Stand Alone system (Tecniplast, PA, USA), which maintains water temperature at 28°C, pH at 7.50–8.50, conductivity at 650–700 µS/cm, and a bio-filter based cleaning system alongside regular automatic changes in water to maintain low nitrate content. We then put the water from this system in a large glass tank (30x15x20 cm; water column height 15 cm), where fish were separated by transparent barriers to create an intermediate stage of acclimation to individual housing without sensory isolation (visual or olfactory). We randomly selected a set of 30 fish from the procured population which we moved here; however, four fish did not survive until the post-isolation data-collection period, so that we had data for 26 fish (12 males and 14 females), We maintained a 12:12 light: dark circadian cycle (from 10 am to 10 pm) in the lab, and fish were fed ad libitum Tetra-Tetramin flakes once daily [11]. We conducted experiments between 10:00 a.m. and 4:00 p.m.

A designated set of 20 fish (10 females and 10 males) was housed together in a separate tank without barriers, using water from the same system. We randomly selected four to serve as the shoal in our social preference tests. We maintained these 20 fish throughout all experiments to ensure consistency in the shoal composition. The ARRIVE and PREPARE international guidelines, as well as CCSEA national and institutional guidelines, were followed for the care and use of animals. We obtained ethical approval from our University's Institutional Animal Ethics Committee. We followed the Guide to Ethical Information Required for Animal Behaviour Papers in the reporting of this manuscript.

Experimental Timeline

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 promptly and 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. We also verified the replicability of metabolic rate measurements by taking repeated measurements on consecutive days on a separate control batch of fish. It is essential to note that, as this is a within-subjects design, all experimental fish were assessed for oxygen consumption and behavioural measures, including social interaction, throughout the study.

Operationalisation and Experimental Design

Social Isolation

During isolation, we moved each experimental fish from its original housing, where it had physical but not olfactory or visual isolation (previously discussed) and placed them

in separate plastic containers (height = 30 cm, diameter = 10 cm, water column height = 15 cm) where we maintained both olfactory and visual isolation. We covered each of these containers with an opaque membrane that prevented the passage of visual cues indicating the presence of conspecifics. Additionally, we used freshly prepared embryo water in these containers during social isolation, allowing us to inhibit olfactory cues and fully standardise the medium each fish experienced. We prepared a stock solution of embryo water of pH 7 to ensure consistency across experiments, which we diluted with fresh distilled water to prepare the isolation medium for each experimental fish (see Appendix I). The embryo water stock solution, with a pH of 7, was prepared according to the guidelines of Cold Spring Harbour Laboratory (2011) [40] and stored in a climate-controlled environment at approximately 28°C. 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. To maintain water quality in the isolation containers, we performed a 50% replacement of the water twice a week, using freshly prepared dilutions of the same stock solution with distilled water. Owing to logistical constraints, we chose 1 month (30 days) as our duration of isolation. This isolation duration allowed us to collect data for an intermediate period, ranging from 24 hours to 6 months, which is the most extensively reported duration of social isolation used in the literature [10] (see Appendix H).

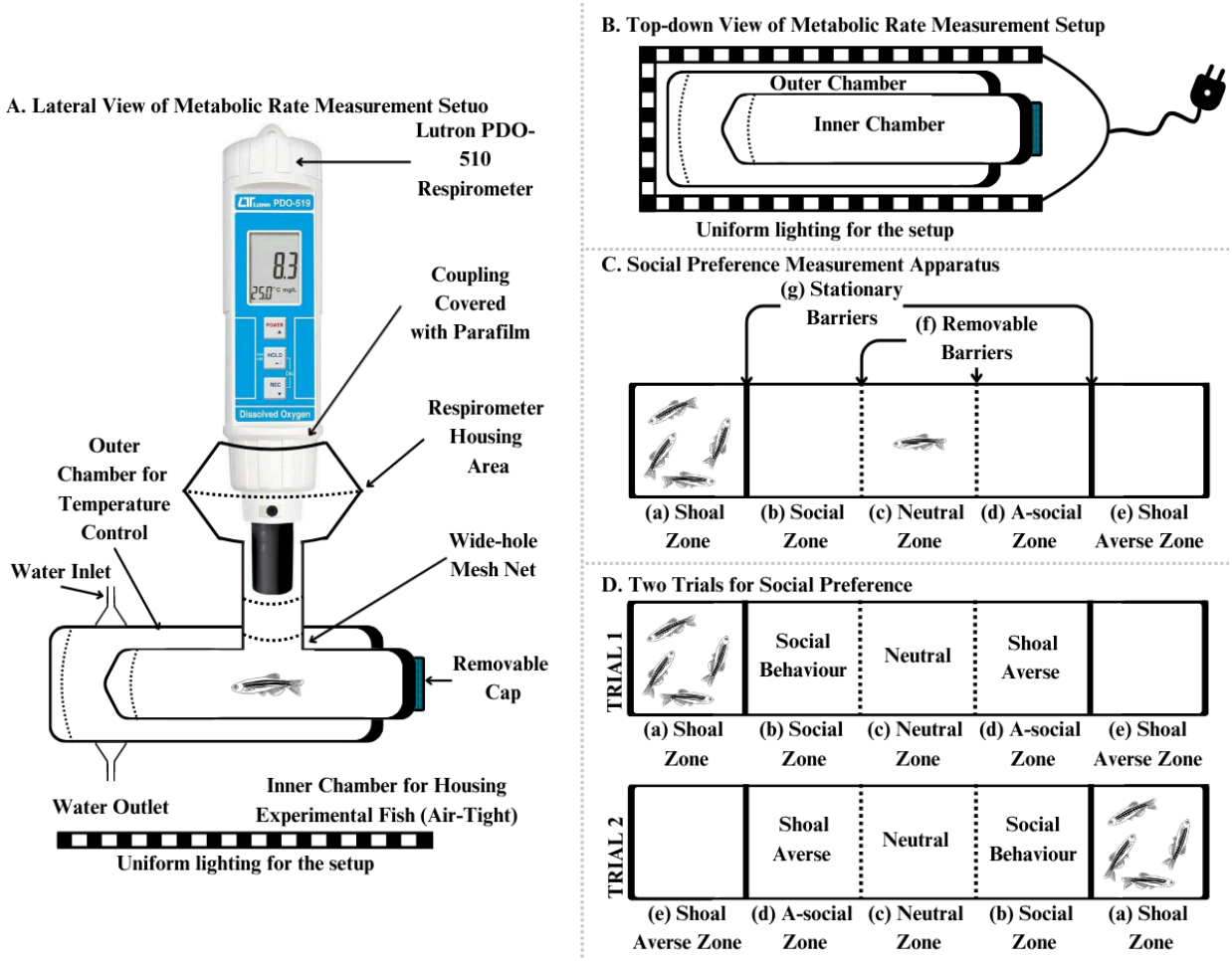

Figure 1: (A) Metabolic Rate Measurement Apparatus, (B) Overhead view of the metabolic rate measurement setup illustrating the lighting provisions, (C) Social Preference measurement apparatus [41], (D) Two-trial setup where we alternated the shoal, showing reversal of behavioural zones.

Metabolic Rate

Our experimental setup, developed in-house, featured a large external chamber that was uniformly illuminated and connected to a temperature control system. A water inlet and outlet facilitated circulation within the outer chamber, maintaining the desired temperature. Inside this outer chamber was a smaller inner chamber with a capacity of approximately 100 mL, which served as the testing environment for the experimental fish.

We introduced an experimental fish into the inner chamber through the upper respirometer housing slot to begin the experiment. Next, we placed a wide-holed net into the same slot to prevent the fish from swimming upward and having direct contact with the probe of our respirometer. Following this, we mounted the respirometer—a Lutron PDO-519 dissolved oxygen meter—into the designated housing, sealing the connection with parafilm to ensure the inner chamber was airtight. This configuration allowed no water exchange between the two

chambers, guaranteeing accurate dissolved oxygen measurements. The variation in dissolved oxygen levels over time directly indicated the oxygen consumption of the experimental fish, thereby providing a means to measure its metabolic rate [42][43][44]. Figures 1A and 1B depict our experimental setup.

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. Therefore, we limited our recording time to a maximum of 90 minutes.

Social Preference

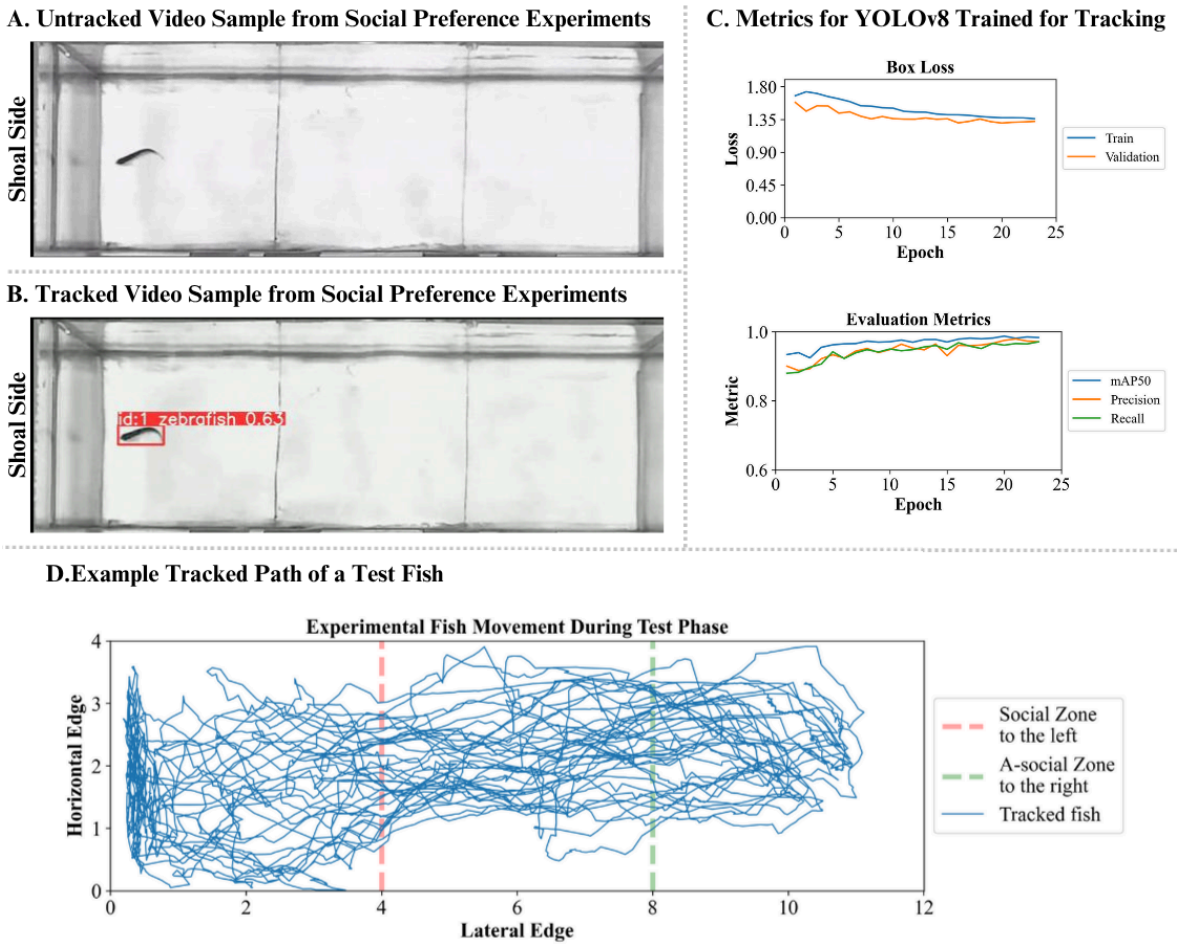

Figure 2: (A) Freeze-frame example of a test fish in the social preference setup, (B) Illustration of YoloV8-based tracking of an example fish, (C) YoloV8 train metrics depicting the choice of early stoppage at metric plateau, and (D) Experimental tracked path of an example fish illustrating full path used to derive downstream metrics.

We considered social preference as the bias to live near and interact with conspecifics, as well as the motivation to identify and approach them [45]. 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, 50x10x12 cm^3 plexiglass tank divided into five equal chambers laterally, as shown in Figure 1C, with a water column height of 10 cm. We used an opaque black membrane to cover all four sides of the tank, preventing external confounding stimuli. The tank consisted of four barriers, two of which were stationary and two were removable, as shown in Figure 1C [46]. The stationary barriers were not watertight, and all fish shared the same water. The shoal, composed of 4 display adult zebrafish, was placed at a terminal zone (a) in Figure 1C, while the opposite end (e) in Figure 1C was left empty. In the acclimatisation phase, we placed the experimental fish in the neutral zone (c) in Figure 1C and allowed them to acclimate for 30 seconds [46]. Following this, during the test phase, we removed the removable barriers (f) in Figure 1C and allowed the experimental fish to explore the tank for 300 seconds [46]. The acclimatisation and experimental phase durations were informed by a review [41] and match the modal durations reported across the 28 studies evaluated. We used a Lenovo 300 FHD 1080P Megapixel webcam [47] 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 1D 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 (d) in Figure 1D. 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), as illustrated in Figure 1D [46]. The second trial was conducted immediately after the first, and the side on which we placed the shoal in the first trial was randomised and counterbalanced with the side used in the second trial. Between trials, we kept the experimental fish in their housing chamber during the pre-isolation stage and in their isolation chamber during the post-isolation stage. We replaced fresh water between tests of individual fish to remove olfactory cues from the previously tested fish.

YOLO-Based Fish Tracking

We converted each experimental fish video into grayscale and cropped it to include only the three central zones (b), (c), and (d), as shown in Figure 1C. Subsequently, the videos were stored as .mp4 files and cropped to a fixed duration of 5 minutes, starting from the moment the removable barriers are raised (f) in Figure 1C, eliminating any extra recording time. Figures 2A and 2B illustrate a single frame from the corrected video. Subsequently, we utilised YOLOv8 and Python to perform object tracking and analysis on the corrected videos.

To train YOLOv8, approximately 100 frames from each recorded video from the test phase were sampled. The sampling process yielded approximately 8,000 frames, annotated using Roboflow [48]. These annotated frames were then split into 70%, 20%, and 10% [49], respectively, for train, test, and validation, and YOLOv8 was trained to identify and localise the experimental fish [50][51]. 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 trade off between train time and performance [52]. Furthermore, we trained the model for 23 epochs to prevent overfitting. Our model achieved 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 social and a-social zones.

Social Preference and Speed of Movement

We used the positional information obtained in the social preference identification extract to extract further the distance covered by each experimental fish during the test phase. We can estimate the average speed of the experimental fish throughout the trials by examining the change in position over time, using this information. We compared the amount of time spent in social and a-social zones before and after social isolation, as well as the average speed of movement before and after social isolation, using paired sample t-tests for normally distributed data and a Mann-Whitney U-test for non-normally distributed data, after verifying data normality using a Shapiro-Wilk test.

Metabolic Rate

We recorded the metabolic rate of the fish every minute for 90 minutes in 26 fish, measured both before and after social isolation. Based on the data normality (checked using a Shapiro-Wilk test), we conducted a paired sample t-test for normally distributed data or a Mann-Whitney U-test for non-normally distributed data. We checked for differences in the paired data measured before and after social isolation at each time point after we placed the fish in the inner chamber of the experimental setup. We conducted all statistical analyses using Python.

Results

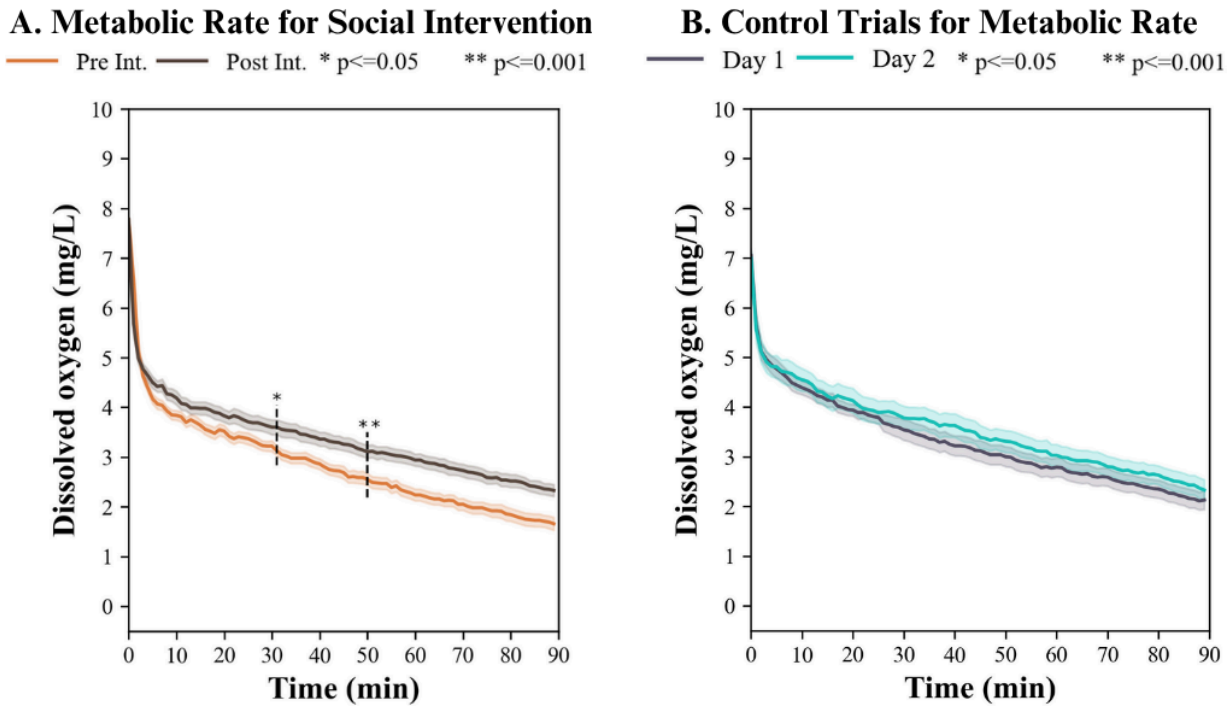

Figure 3: (A) Higher dissolved oxygen level in the metabolic rate recording setup after the administration of social isolation, indicating a lower metabolic rate. There was a statistically significant difference ($p < 0.05$) between oxygen consumption pre- and post-isolation (B). No significant difference at any time point between the metabolic rate recordings carried out on consecutive days (Pearson's correlation coefficient: 0.996, $p \leq 0.05$).

The rate of oxygen consumption after a month-long period of social isolation was significantly lower than the pre-isolation stage at the population level (Mann–Whitney U test, $p<0.05$, $d=-0.550$, N=26: 12 male fish and 14 female fish), as shown in Figure 3A. This decrease in the oxygen consumption rate indicated a lower metabolic rate post-isolation (see Appendix A).

The rate at which fish consumed oxygen in water trials on the two consecutive days was replicable for the metabolic rate control experiment. There was no significant difference observed between oxygen consumption rate on both days for the overall population (Mann–Whitney U test, $p>0.05$, $d=-0.267$, N=15: 9 male fish and six female fish), as shown in Figure 3B (see Appendix G). Batch identity influenced the baseline measure; however, repeated measures showed a significantly high correlation between measurements across days for a given batch of fish. (Pearson's correlation, $r= 0.996$, $p<0.05$, $d= -0.267$, N=15: 9 male and six female fish).

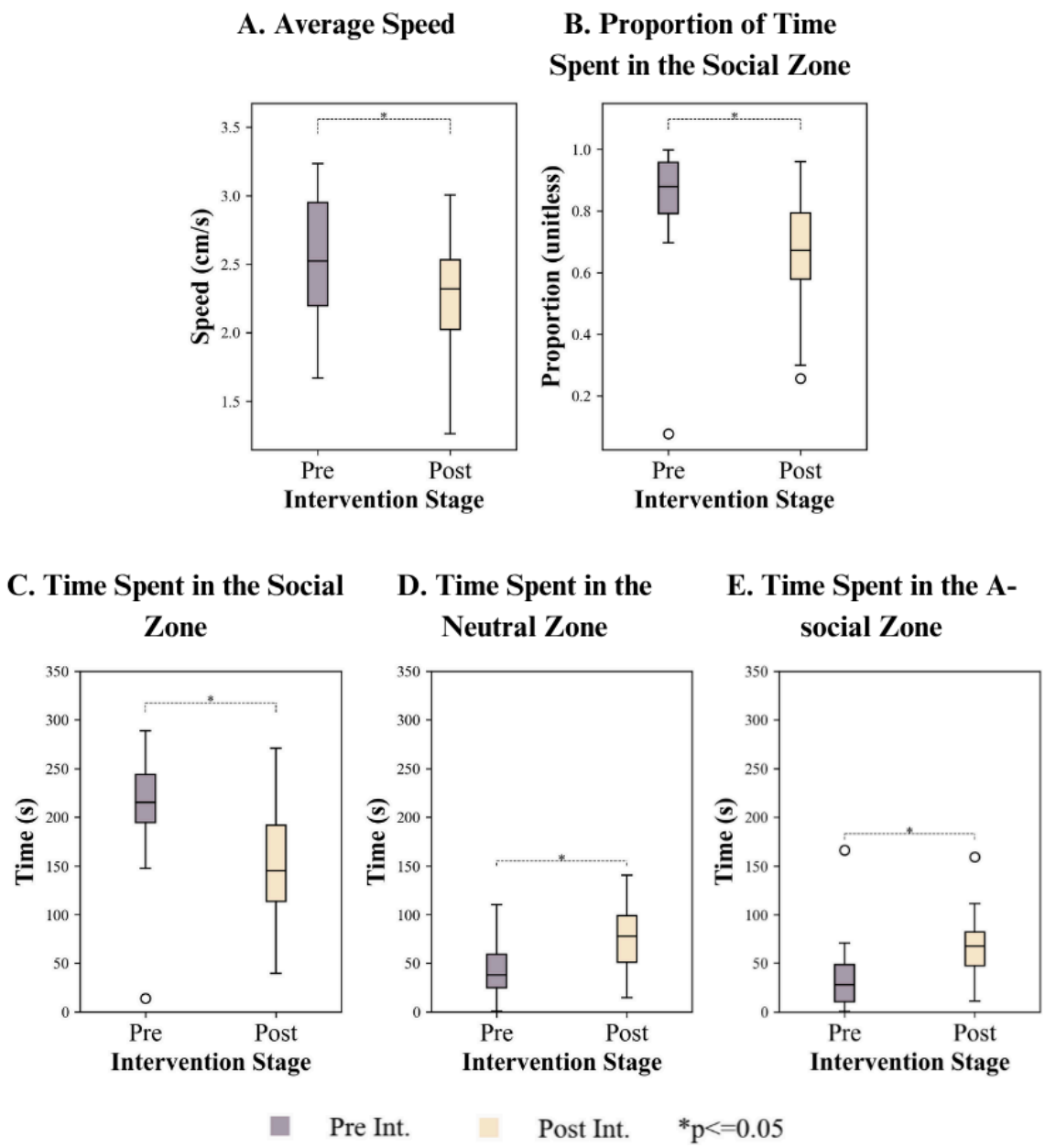

Figure 4: (A) The average speed of movement (cm/s) of the fish significantly ($p<0.05$) reduced after social isolation, (B) The proportion of time the fish spent in the social zone was significantly less after social isolation, compared to the pre-isolation stage, (C) There was a statistically significant decrease ($p<0.05$) in time spent by the fish in the social zone post-isolation, (D) indicated by a statistically significant rise ($p<0.05$) in the time spent by the fish in the neutral (E) and a-social zones (F).

The one-month isolation treatment also significantly reduced the average speed at which the fish travelled post-social isolation (M=2.22, SD=0.425) compared to the pre-social isolation stage (M=2.520, SD=0.472) at the population level (Paired sample t-test, $p<0.05$, d=0.677, N=26: 12 male fish and 14 female fish), as shown in Figure 4A (see 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-isolation stage (M=0.843, SD=0.183), at the population level (Mann-Whitney U test, $p<0.05$, d=1.009, N=26: 12 male fish and 14 female fish), as shown in Figure 4B (see Appendix C). This period of social isolation also significantly reduced the absolute time spent by the fish in the social zone post-isolation (M=152.17, SD=61.76) when compared to the pre-isolation stage (M=213.52, SD=58.37) for the overall population (Mann–Whitney U test, $p<0.05$, d=1.041, N=26: 12 male fish and 14 female fish), as shown in Figure 4C (see Appendix D).

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Conversely, there was a significant increase in the time spent by the fish in the a-social zone post-isolation (M=68.97, SD=31.86) when compared to the pre-isolation stage (M=34.33, SD=34.41) at a population level (Mann–Whitney U test, $p<0.05$, d=-1.065, N=26: 12 male fish and 14 female fish); as well as in the neutral zone post-isolation (M=74.90, SD=35.81) when compared to pre-isolation (M=41.59, SD=27.58), for the overall population (Paired sample t-test,$p<0.05$, d= -1.062, N=26: 12 male fish and 14 female fish), as shown in Figures 4D and 4E (see Appendix E and F). Our results indicated a reduction in overall social interaction, or a decrease in the experimental fish's preference for interacting with conspecifics, after one month of social isolation. Additionally, we evaluated the effect of sex differences on the changes in metabolic rate and found no sex-specific variation in metabolic rate or social preference.

Discussion

Our study examined oxygen consumption and social preference in wild-type zebrafish to investigate the contextual advantages of social isolation-induced reductions in movement. Our study contributes to the existing literature by quantifying oxygen consumption accompanying behavioural changes in social preference and movement rate induced by social isolation. After one month of social isolation, we report reduced oxygen consumption and decreased social preference, accompanied by lower movement rates. This approach offers insights into understanding the bioenergetic state of an organism when it is 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 [19][53], the observed reduction in movement, social interaction, and metabolic rate in socially isolated fish effectively demonstrates a potentially energetically advantageous decrease in energy expenditure. Past literature has looked at some of these variables separately but found results that corroborate ours: varied activity levels across various species of fish (bonefish, cunner, carp, roach) correlate with differences in energy expenditure [54], and the energetic costs of swimming [19] are higher than the energetic costs of simply being still [55], supporting our overall finding that lowered movement lowers energy expenditure [19][56][57][2].

However, in resource-limited ecological contexts, reduced locomotion, such as shorter or less frequent foraging trips, has been shown to prioritise energy conservation, often resulting in lower overall energy intake and adult body mass, as seen in pelagic seabirds [58]. Similarly, in zebrafish, reduced locomotion may constrain foraging activity and consequently diminish overall energy intake under such conditions [59]. Furthermore, evidence from other teleosts indicates that energy status modulates social tendencies: food-deprived juvenile qingbo carp (*Spinibarbus*

sinensis) display reduced sociability, prioritising foraging over social interaction, whereas well-fed individuals with higher metabolic rates tend to limit social contact to minimise competition (Killen et al., 2016). Thus, social withdrawal could allow one to repurpose metabolic savings for various bodily functions, such as reproductive or immune investment. While these findings provide practical comparative context, the present study did not directly assess food intake or energy storage, and such interpretations therefore remain speculative. Future investigations should examine whether measurable changes in feeding behaviour, metabolic expenditure, or motivational states accompany visual and olfactory isolation in zebrafish. Although some studies have suggested that social isolation may influence gamete quality in zebrafish [60][61], broader assertions regarding reproductive or immunological advantages should be made cautiously, given interspecific variation in social structure and ecological pressures.

Socially withdrawn crustaceans showing reduced activity levels also encounter fewer predators than crustaceans swimming in shoals, reducing their chances of being preyed on by Stickleback [62]. This reduction is noteworthy in environments with high predatory threats. Hence, a reduction in the 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. We aim to conduct further experiments to collect standardised data and determine the reproduction rate after social isolation, thereby gathering replicable data to facilitate definitive conclusions.

Zebrafish show behavioural changes similar to those seen in humans after being socially isolated for a specific period [2][63]. We quantified these behaviours in our study and confirmed earlier literature on the effects of social isolation in chronically and acutely isolated adult zebrafish. Socially isolated zebrafish exhibit a decrease in overall movement and foraging behaviour, reduced interest in exploring the environment, and a poor preference or motivation for social interaction [64][10][65].

The 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 [66]. When the immune system responds to pathogens, it triggers inflammatory reactions that can affect the nervous system [67], including reduced movement, which in turn lowers the risk of infection by other conspecifics within the social group [68].

Zebrafish that were raised in complete isolation since birth, when tested in adulthood, demonstrated significantly less social preference when compared to socially raised fish [65]. We therefore found that social isolation did not drive social interaction but increased social aversion. The period of isolation appears to be significant: adult zebrafish isolated acutely for 24 hours did not exhibit changes in locomotor activity or social preference when exposed to their conspecifics [10], whereas those isolated for a prolonged period of 6 months showed significant changes in locomotor activity and reduced social preference [10]. Thus, we inferred that increased social isolation leads to a decrease in social preference and is associated with changes in locomotor activity. We replicate our results, finding that the effects of social isolation for one month are similar to those 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 [69][70]. 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 a result of social isolation [71][72].

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 [73][74]. Further, keeping the fish in an airtight chamber for 90 minutes could also have induced hypoxia, another potential stressor [75]. Although the chamber had a 100 mL capacity, the actual volume of water used was slightly less due to displacement and the setup of the equipment. The central tube had a diameter of 3.7 cm, which is adequately larger than the average adult zebrafish length of 2.8 cm, allowing unrestricted turning and natural horizontal swimming. The dorsal fin remained fully submerged throughout, minimising physical discomfort. However, we acknowledge that the confined space may have posed a mild stressor. While we observed no overt signs of distress or hypoxia, we did not explicitly measure physiological stress markers such as cortisol levels or oxygen saturation. Future studies should assess stress or hypoxia indicators to confirm the tolerability of prolonged confinement in similarly sized chambers. Another potential limitation is that the experimental fish may have briefly seen the experimenter's hands when removing the removable barriers from the social interaction setup. Although we took care to standardise this procedure across trials using consistent hand movements and timing, visual exposure to the experimenter and sudden water movement may have introduced a mild, uncontrolled stimulus [76]. While the brief exposure was unlikely to have significantly influenced behaviour, future protocols could incorporate visual shielding or automated barrier removal to eliminate this variable.

Additionally, it is essential to note that the isolated fish were exposed to their conspecifics, visually and olfactorily, during the social preference test performed before isolation. Furthermore, the novel environment created by the social preference set up after 30 days of social isolation, alongside the presence of other fish, could have induced stress in the tested fish [76], which may have impacted post-isolation metabolic rate measurements. That said, we also anticipated some stress reduction due to the fish experiencing some relief from the visual and olfactory isolation [15][77]. Anticipating that the metabolic assay could be stressful, we prioritised evaluating its effects on control fish over two consecutive days. Finding no difference there, and only differences within socially isolated fish between day 1 and day 30 of isolation, we ascertain that isolation alone could explain the outcome change.

To conclude, this study quantified metabolic activity, social interactions, and movement patterns to identify parameters relevant to understanding the bioenergetic correlates of behavioural changes induced by social isolation in wild-caught zebrafish in specific contexts. Reduced energy expenditure has several context-specific advantages that could improve the chances of survival by increasing reproductive fitness. This study opens up numerous possibilities for exploring depressive behaviours within a wide range of simulations that accurately mimic specific ecological frameworks to examine the adaptive significance of these traits.

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, and SR wrote the manuscript.

Competing Interest

The authors declare no competing interests.

Additional Information

We followed CCSEA 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

Data and code are available at our project GitHub Repository found at https://github.com/mraoaakash/EcoAdvDep

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Appendices

Appendix A

Social isolation: Metabolic Rate before and after isolation

	Cohen's *d*
Pre-isolation	-0.555
Post-isolation	-0.555

Appendix B

Social isolation: Average speed comparison before and after isolation

	Shapiro-Wilk (W)	Shapiro-Wilk (p)	Mean	Standard Deviation	Cohen's *d*	*T*-Test (p)
Pre-isolation	0.9506	0.240	2.52	0.472	0.677	0.013
Post-isolation	0.9517	0.255	2.22	0.425	0.677	0.013

*$p<0.05$

Appendix C

Social isolation: Proportion of time spent in the shoal zone before and after isolation

	Shapiro-Wilk (W)	Shapiro-Wilk (p)	Mean	Standard Deviation	Cohen's *d*	Mann-Whitney *U*	Mann-Whitney (p)
Pre-isolation proportion	0.6841	3.24E-06	0.8433	0.18349	1.009	554	8.02E-05
Post-isolation proportion	0.9664	0.5320	0.6636	0.1796	1.009	554	8.02E-05

*$p<0.05$

Appendix D

Social isolation: We compare the time spent in the social zone before and after the isolation period.

	Shapiro-Wilk (W)	Shapiro-Wilk (p)	Mean	Standard Deviation	Cohen's *d*	Mann-Whitney *U*	Mann-Whitney (p)
Pre-isolation	0.8776	0.005	213.52	58.37	1.041	529	0
Post-isolation	0.9777	0.821	152.17	61.76	1.041	529	0

*$p<0.05$

Appendix E

Social isolation: Time spent in the neutral zone comparison before and after isolation

	Shapiro-Wilk (W)	Shapiro-Wilk (p)	Mean	Standard Deviation	Cohen's *d*	*T*-Test (p)
Pre-isolation	0.9619	0.429	41.59	27.58	-1.062	0
Post-isolation	0.9603	0.399	74.90	35.81	-1.062	0

*$p<0.05$

Appendix F

Social isolation: Time spent in the a-social zone comparison before and after isolation

	Shapiro-Wilk (W)	Shapiro-Wilk (p)	Mean	Standard Deviation	Cohen's *d*	Mann-Whitney *U*	Mann-Whitney (p)
Pre-isolation	0.7798	8.14E-05	34.33	34.41	-1.065	118	5.89E-5
Post-isolation	0.9632	0.4596	68.97	31.86	-1.065	118	5.89E-5

*p<0.05

Appendix G

Metabolic Rate Water Control Trial Results

	Cohen's *d*	Pearson's (r-value)	Pearson's (p-value)
Day 1	-0.267	0.996	1.64E-95
Day 2	-0.267	0.996	1.64E-95

Appendix H

Social Isolation Protocol:

Pre-isolation

Task (check two fish per day)	Method
Social Interaction	1. Each zebrafish was individually introduced to the central zone of the testing apparatus and was temporarily separated by a transparent dividing door that could be slid up. This door divides the central zone from the left and right arms of the corridor. 2. 30 seconds of acclimatisation in the central part with the glass doors shut. 3. Following the initial 30-second acclimation interval, the two sliding dividers should be gently lifted, and the zebrafish should be released to explore the apparatus for 5 minutes. 4. Alternate the side on which the fish shoal was put and conduct the trial for the target fish again.
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*. Check the reading on the respirometer every minute and note it down. 6. Take readings for 90 minutes.
Feeding	ad libitum Tetra-Tetramin flakes once daily

Post isolation

Task	Method
Social Isolation isolation	1. Use opaque white containers, fill them with embryo water, and keep one fish per container. 2. 2 fish were socially isolated each day
Social Isolation	30 days

Social Interaction	1. Each zebrafish was individually introduced to the testing apparatus's central zone, temporarily separated by a transparent dividing door that could be slid up. This door divided the central zone from the left and right arms of the corridor. 2. 30 seconds of acclimatisation in the central part with the glass doors shut. 3. Following the initial 30-s acclimation interval, the two sliding dividers should be gently lifted, and the zebrafish should be released to explore the apparatus for 5 min. 4. Alternate the side on which the fish shoal was put and conduct the trial for the target fish again.
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. Check the reading on the respirometer every minute and note it down. 6. Take readings for 90 minutes.
Feeding	ad libitum Tetra-Tetramin flakes once daily

Appendix I

Procedure to make embryo water:

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 a 60x stock developed by the Cold Spring Harbour Laboratory (2011) for the use of zebrafish embryos:

a) Dissolve the following salts in 2L of H20 : 34.8 g NaCl; 1.6 g KCl; 5.8 g $CaCl_2 \cdot 2H_2O$; 9.78 g $MgCl_2 \cdot 6H_2O$.

b) Adjust the pH to 7.2 with NaOH.

c) Autoclave.

d) To prepare 1X medium, dilute 16.5mL of the stock to 1 L

e) Add 100 µL of 1% methylene blue (Sigma-Aldrich)

The 1X solution will be prepared freshly from the stock as needed.