Development of a Raspberry Pi-based Automated Fish Training Apparatus

1. Abstract

The development of RaspberryPi based automated fish training apparatus aims to revolutionize the traditional methods of fish training and behaviour by combining modern technology with behavioural research. This thesis presents the design, implementation and evaluation of a fish training system which utilizes Raspberry Pi's computational capabilities to provide real-time tracking and analysis of fish movement, response to stimuli and learning progress. The thesis aims to evaluate the effectiveness of Raspberry Pi-based automated fish training apparatus in training wild fishes, particularly cichlids, which show highly developed socio-cognitive abilities and show highly developed behavioural responses to the environment, heterospecifics and their group mates. This thesis also aims to develop a network of such apparatus to standardize data collection, conduct comparative studies across different age groups, sex and species, and optimization of training protocols for maximizing learning and behavioural outcomes.

2. Background

The evolution of intelligence and the evolutionary processes that shaped cognitive traits have mystified biologists since Darwin proposed the theory of natural selection, and it remains one of the major themes in behavioural biology. Fish is one of the vertebrate groups with a vast diversity in morphology, ecology and life-history traits. Moreover, it has been established that the cognitive abilities of fish are complex, diverse and comparable to humans and other terrestrial vertebrates. It has been proved that fish possess long-term memory, can recognise motion patterns, learn through their companions, and cooperate both within and between species making them a suitable model for evolutionary ecology and animal cognition.

Colour and shape perception allows an organism to discriminate its visual environment and is involved in many fitness and survival-related activities such as avoiding predation, finding food resources and mates, defence, and competing with rivals. Like other traits, cognition has an adaptive significance and undergoes natural selection. The evolution of cognition and selection requires some heritable differences between individuals. Behavioural and social traits such as sociability, altruism, aggressiveness, sexual preferences, and parental behaviours are some heritable differences that provide prerequisites for investigating the evolution of cognition. Intraspecific variation in cognition can explain the evolution of cognitive abilities. To fully understand how cognitive abilities differ by age, sex, and individual morphological variations, we need a comprehensive study to research on vision, perception and numeric abilities of fish.

African cichlids provide a great model for understanding cognition due to their diverse and complex behaviours, well-developed brains and genetic tractability. Cichlids exhibit territoriality, courtship rituals, cooperative breeding and parental care, suggesting the presence of sophisticated cognitive abilities relating to decision-making and social interactions (Balshine-Earn S et al, 1998). Cichlids have demonstrated the ability to learn and memorize tasks in lab settings, making them a model to study learning and memory processes. Cichlid brains have specialized regions that control various functions like decision-making, problem-solving and spatial memory (Maruska, 2012). Cichlids are also one of the most diverse fish families and have adapted to varying environments making them a very relevant and suitable model for studying the evolution of social cognition in natural habitats. (Canestro et al., 2016).

One major difficulty in conducting a large-scale cognition research project is to test a very large number of fish in several learning tasks. Testing individuals in multiple tasks is necessary as performance in one task can be unrelated to performance in another. However, manual testing and training fish individually for these experiments limit the number of fish to be sampled per day. These learning routines also require time prior to the experiment to produce adequate sample sizes leading to laborious and expensive experiments which are also vulnerable to various confounding biases like subject-observer bias. Automated conditioning devices could ameliorate these limitations in cognition experiments.

The development of an automated conditioning apparatus standardizes and automates the process of training and testing animals in controlled environments and studies various aspects of cognition and behaviour with precision and reproducibility. These devices can implement complex experimental paradigms and ensure that testing conditions are consistent across different species and experiments. Standardization in testing increases the reproducibility of research and enhances the accurate replication of experiments and validation of the results. The automated conditioning apparatus facilitates precise control on various environmental factors, elimination of confounding variables, and ensure adherence to ethically sound protocols. Automated systems provide objective measurements of behaviour, reducing the potential human biases and minimizing subjective interpretations while simultaneously allowing high throughput screening to efficiently analyze large datasets.

3. Literature Review

Gatto et al., 2021 developed the automated operant conditioning procedure and compared it with manual routine training procedures by training laboratory-bred adult female guppies. The apparatus was controlled by a microcomputer, which displays stimuli on a monitor, tracks the movements of the fish, and delivers a very small amount of food when the subject makes a correct response. Each subject underwent the habituation phase, pre-training, training and testing phase. The habituation procedure familiarized guppies with the experimental apparatus, and pre-training helped them familiarize themselves with the experimental procedure. The subjects were trained on numerical contrasts, and the difficulty of tasks was progressively increased until the fish could no longer reach the testing criterion. The results of this study confirmed that automated training devices modelled on the Skinner box were successful in training guppies in some cognitive tasks such as colour, shape and size discriminations but were inefficient in numerical discrimination. Another attempt was made by Ajuwon et al, 2021 through their platform GoFish. They developed the Bonsai custom workflow, which used HSV thresholding to detect Goldfish. Bonsai and Bonvision were used for stimuli generation, and the experimental routine involved pre-training, training for acquisition and reversal of spatial conditioning, and colour discrimination. The experiment showed that Goldfish were successfully trained to associate a fixed location with a reward, reverse their preference, and colour discrimination. Aoki et al., 2015 automated a Y-maze avoidance task for adaptive learning in Zebrafish using custom-built MATLAB GUI. Fish was detected using background subtraction in grayscale images. The zebrafish showed a higher success rate in learning rate than similar manual behavioural paradigms. In another experiment, Babkiewicz et al., 2021 employed an automated T-maze where video recording and stimuli presentation was facilitated by ViewPoint's application ZebraLab Tracking. The experiment was successful where fish likely learned the association of colour with food, and the results demonstrated that temperature enhanced the spatial learning rate in Zebrafish. Brock et al., 2017 developed a fully automated Skinner box to test for Pavlovian fear conditioning and Five-choice serial reaction time and were able to train adult guppies with accuracy exceeding 70% in three weeks.

Through the cited sources, it is clear that automated apparatus have shown success in fish learning assay. However, one of the major limitations of these experiments is that none of these experiments are conducted on cichlids. Cichlids exhibit complex social behaviour and cognitive abilities such as territoriality, courtship, aggression, and parenting and are well-suited to study social cognition and the neural basis of social interactions. Demonstrating that cichlids could be trained using automated apparatus for simple colour, shape and numerical discrimination tasks could open the avenue to use automated conditioning and testing apparatus in complex social cognition and collective behaviour experiments. Another drawback is that none of these studies are conducted on wild fishes. The intensity of several complex social behaviours, such as forming hierarchies, territoriality, and courtship rituals, is reduced in domesticated cichlids compared to wild cichlids. Wild cichlids also display remarkable behavioural plasticity and rapidly adjust its behaviour and cognitive strategies in response to a change in environment and social status (Maruska, 2013). Thus, the success of automated apparatus in training wild fishes could make conducting research on fish in their natural habitat more convenient and could result in novel insights into natural behaviours and adaptations. One more limitation is that the automated training apparatus described does not automatically switch from pre-training to training and increase the difficulty level of discrimination based on the fish response. This leads to inevitable interaction between the experimenter and the fish when a new training regime is introduced. Moreover, the development of a network of such apparatus allows for centralized data collection, storage and management with comprehensive information enabling data standardization across different subjects and could be further employed to study different species simultaneously.

4. General Goals of Thesis

Keeping in mind the present limitations of automated conditioning and testing apparatus, this thesis aims the following:

a. Develop a low-cost, robust fish tracking and conditioning apparatus that minimizes the experimenter-subject interactions during behavioural experiments
b. Development of a Client-server architecture to improve existing data consolidation and management pipelines in automated cognition testing
c. Investigate the success of automated devices in training wild fish.

5. Proposed Methodology

The apparatus would consist of a microcomputer, feeding device, the tank, which would also house the fish, and LCD Display screens which would show the stimulus. The device would be controlled by a microcomputer, Raspberry Pi 4B installed with Raspbian 64-bit OS, which would track the position of the subject in the tank and record the experiment. It would also control the display of stimuli on LCD Screens, dropping of food rewards through fish feeders. The detection of Cichlids would take place through a custom-trained YOLOv5s model deployed on Raspberry Pi. The Graphical-user-interface (GUI) would be developed using PyQT5 as it provides a range of classes and tools for building interactive and feature-rich desktop applications. Further, using Socket, a Python library, a Client-Server architecture can be developed where one RaspberryPi (server) sends instructions to other RaspberryPis (clients) on executing the experiments, storing and comparing the final results of each apparatus for comparative studies.

The apparatus will be used to train wild *Astatotilapia burtoni* found in Lake Tanganyika, Zambia. To maintain the optimum conditions of water, the tank can be fitted with a heater and filter. However, in case of limited power supply in the fieldwork, some fraction of water could be replaced daily to ensure optimum rearing conditions are maintained for fish throughout the experiment. The tank would be divided into starting area, corridor and Region of choice, as shown in Figure 1. Three identical apparatus would be used at the same time and would be placed adjacent to each other in a sunlit room. The experiment protocol is described as follows:

a. **Habituation:** In this phase, the subject is accustomed to the tank and its new surroundings. The experimenter enters the number of days the habituation phase will last and the number of food rewards given. This phase will have no stimuli presentation, but food rewards will be given in regular intervals in the Region of choice. The experimenter would set the criterion on the number of food rewards consumed by the subject in subsequent sessions for the subject to be considered fit for pre-training and training routines.

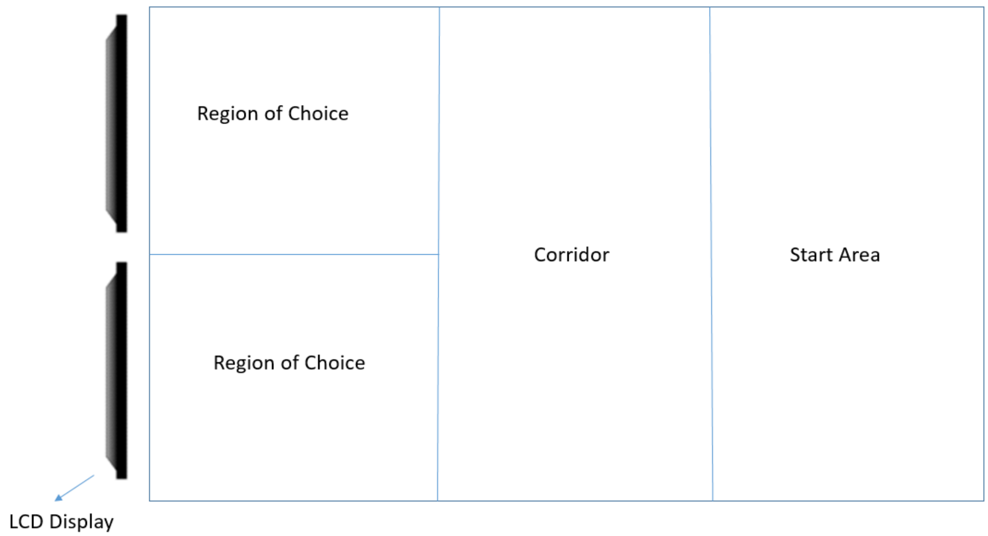

Figure 1: Schematic representation of three regions in which tank is divided along with LCD displays to present stimuli

b. **Pre-Training:** During the pre-training phase, subjects would be habituated to the training procedure. The experimenter would set the number of days for which pre-training needs to be conducted and the number of sessions taking place each day. The experimenter would also set up values like stimulus display time, the number of food rewards needed to be consumed for a session to be successful, the maximum duration of a session, the interval between two sessions, the maximum number of sessions etc. As shown in Figure 2, the experiment would begin once the subject is in the start area. The subject's presence in the start area would be followed by a display of stimuli. If the subject enters the Region of Choice, the food reward will be dropped. A trial would be considered successful once the fish starts from the choice area, enters the Region of choice, consume food reward and then comes back to the start area. A session would end if the maximum duration of the session is passed or the subject completes the maximum number of trials as specified by the experimenter. A session will be said successful if the subject consumes a specified number of food rewards. If the subject consumes the specified number of food rewards in the number of consecutive sessions specified by the experimenter, then the subject would be deemed as pre-trained. Otherwise, the pre-training would go on until the subject meets the criterion for pre-trained or the limit to maximum number of sessions for pre-training is reached. If the fish is not able to meet the pre-training criterion in the maximum number of sessions, it would not be considered fit for training experiments.

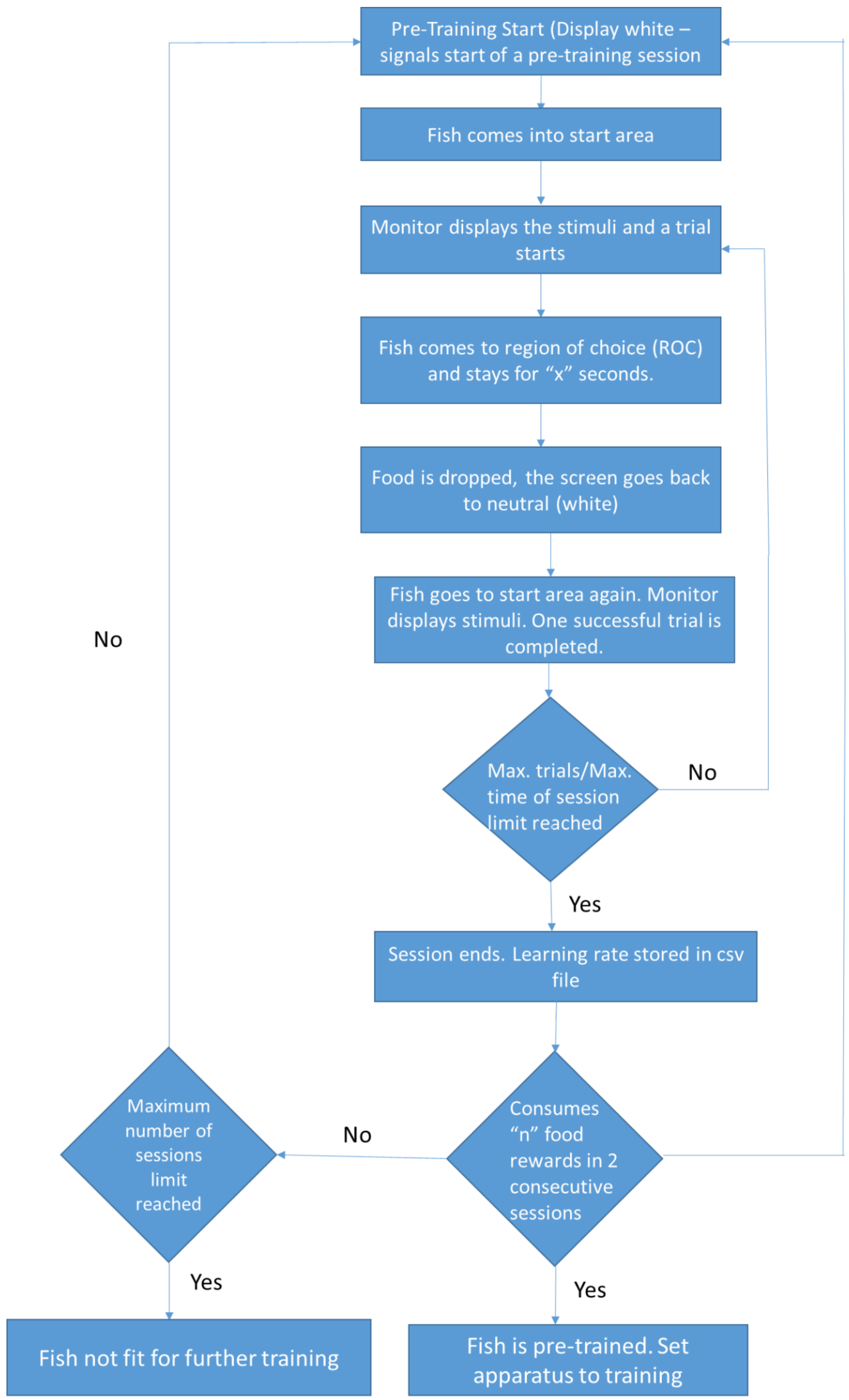

Figure 2: Flowchart illustrating Pre-Training process

c. **Training:** In the training, the subject would be presented with different levels of discrimination, with the difficulty level of discrimination increasing as the subject learns to discriminate between choices presented in the previous levels. Similar to pre-training, the experimenter would specify the number of days for which training needs to be conducted, the number of sessions taking place each day, stimulus display time, the maximum duration of the session, the interval between two sessions, the maximum number of sessions, learning criterion of a session ((number of successful trials/Total number of trials)*100) etc. The experiment would begin once the subject is in the start area. The subject's presence in the start area would be followed by a display of two choice stimuli on the LCD Screen. The appearance of choices on the screen would be randomized after every trial, ensuring that the correct choice does not consecutively appear more than thrice in the same choice area. If the subject enters the Region of Correct Choice (ROCC) and stays for a specified duration of time, the food reward will be dropped, and the screen will turn back to a neutral colour. A trial would be considered successful if the fish enters the Region of Correct choice, consumes the food reward and then comes back into the start area. The session ending criterion remains the same as pre-training. If the subject reaches the learning criterion in a session, the session will be considered successful. If the subject reaches the learning criterion in a consecutive number of sessions as specified by the experimenter, then the subject would be considered trained in discriminating the given pair of stimuli, and the apparatus would shift to the next difficulty level of training.

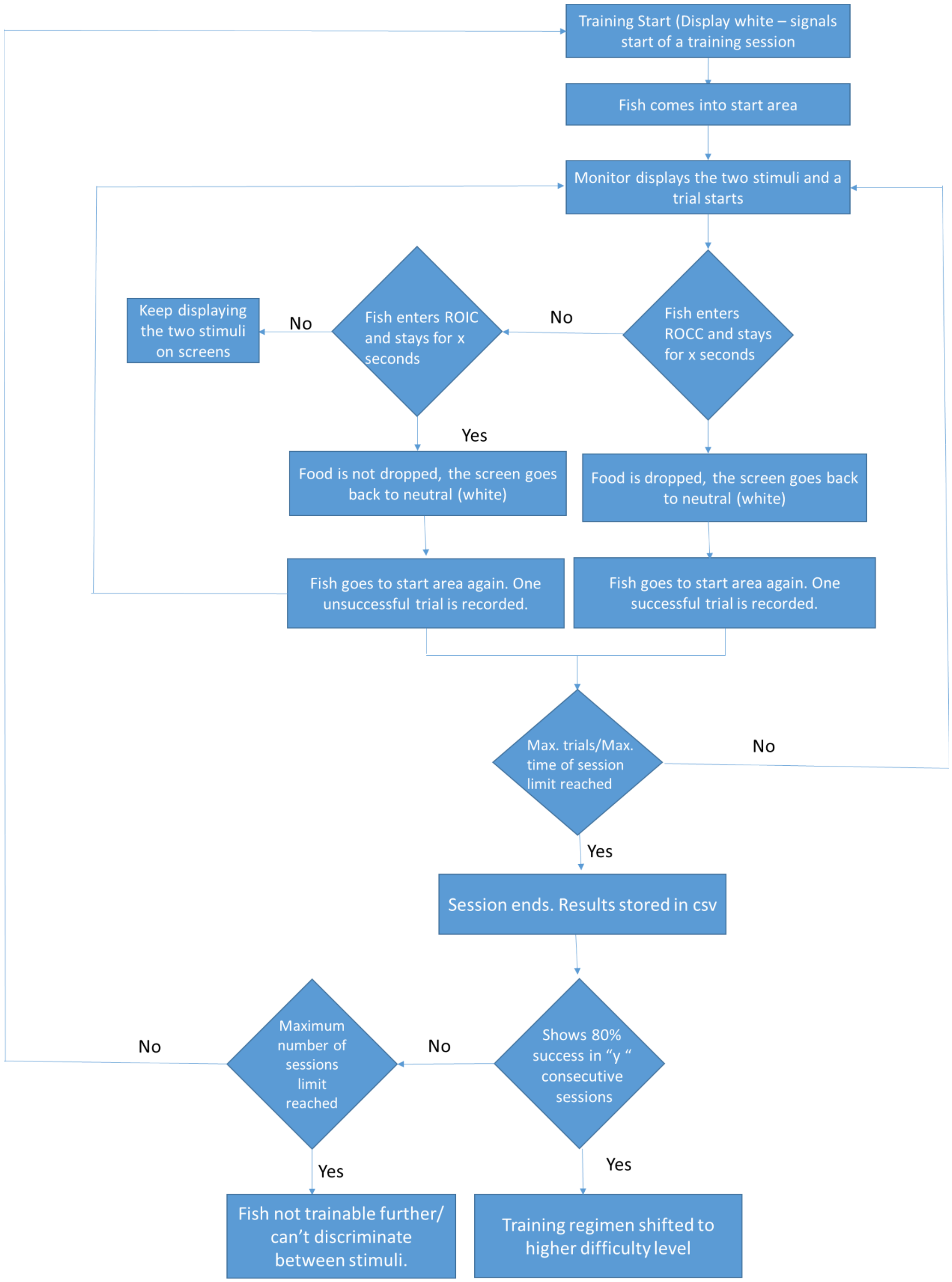

Figure 3: Flowchart illustrating Training process

7. Timeline

S.No	Tasks	Timeframe
1.	Implementation of experiment recording, subject detection and tracking, and presentation of stimulus	July
2.	Coding for Habituation, Pre-Training, Training Routine	August
3.	Development of GUI and Wireless Networks of Raspberry Pi Controlled Apparatus	September
4.	Testing the apparatus in the field	October-November
5.	Data Analysis of Results	December

8. References

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