INVESTIGATION OF THE EFFECTS OF SENSORY SALIENCE ON THE REFUGE TRACKING PERFORMANCE OF WEAKLY ELECTRIC FISHES

ZAYIF ELEKTRİK BALIKLARINDA ALGISAL BELİRGİNLİĞİN SIĞINAK TAKİBİ BAŞARIMINA ETKİLERİNİN İNCELENMESİ

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ABSTRACT

INVESTIGATION OF THE EFFECTS OF SENSORY SALIENCE ON THE REFUGE TRACKING PERFORMANCE OF WEAKLY ELECTRIC FISHES

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Weakly electric fish species typically demonstrate hiding behaviors among plant roots and suspended objects in streams within their natural habitats. These fish employ the force generated by their anal ribbon-like fins to facilitate back-and-forth movements, thus modulating visual and electrosensory feedback. These movement abilities allow them to effectively maintain their position in a computer-controlled moving refuge. For our research, we conducted behavioral experiments involving wave-type gymnotiform species, namely, *Apteronotus albifrons* called black ghost knifefish and *Eigenmannia virescens* called glass knifefish, native to South America. We utilized an experimental setup consisting of a 3D-printed refuge, the motion of which was controlled using a linear actuator. The 3D refuges, made of PLA material, were prepared in three different lengths (7 cm, 14 cm, and 21 cm) and two surface feature variations (with and without windows). The refuge was moved by the sum of sinuses in the range of 0.10 Hz to 2.05 Hz with the help of a special code on the computer. The experimental conditions encompassed manipulations of four variables: 1) lighting levels (dark, dim, and light), 2) refuge structure (with or without windows), 3) refuge length (7 cm, 14 cm, 21 cm), and 4) conductivity levels (low, medium, and high). A total of 54 different sensory conditions were tested, (with N=5 individual fish) for each species. There was a statistically significant difference between species; with black ghost knifefish having lower RMSE values than glass knifefish species. The most important parameters for refuge tracking performance were illumination and length. Moreover, the conductivity had a significant impact on tracking performance. However, we determined that the window condition did not have the same effect on refuge tracking performance. Besides, we found statistically significant interactions between the 'Species and Length', 'Species and Window', 'Illumination and Length', 'Illumination and Window' conditions. The length of the refuge had a greater effect on the refuge tracking performance of the fish than whether the refuges are windowed or not. The presence of windows opened in refuges in well illuminated conditions had a minor effect on tracking performance, while the presence of windows in refuges in dark conditions had a positive effect on tracking performance, making a big difference in results. The results showed that refuge tracking behavior was much more successful at low conductivity. Fish weighted visual information when electrosensory salience was compromised at high conductivities.

Keywords: weakly electric fish, system identification, sensory salience, tracking behavior, active sensing

ÖZET

ZAYIF ELEKTRİK BALIKLARINDA ALGISAL BELİRGİNLİĞİN SIĞINAK TAKİBİ BAŞARIMINA ETKİLERİNİN İNCELENMESİ

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Zayıf elektrikli balık türleri, doğal yaşam ortamlarında tipik olarak bitki kökleri ve akarsulardaki asılı nesneler arasında saklanma davranışları gösterir. Bu balıklar, ileri geri hareketleri kolaylaştırmak için anal şerit benzeri yüzgeçlerinin ürettiği kuvveti kullanır, böylece görsel ve elektrosensörsel geri bildirimi modüle eder. Bu hareket yetenekleri, bilgisayar kontrollü hareketli bir sığınakta konumlarını etkili bir şekilde korumalarına olanak tanır. Araştırmamız için, Güney Amerika'ya özgü, siyah hayalet bıçakbalığı olarak adlandırılan *Apteronotus albifrons* ve cam bıçakbalığı olarak adlandırılan *Eigenmannia virescens* gibi dalga tipi gymnotiform türleri içeren davranış deneyleri gerçekleştirdik. Hareketi doğrusal bir aktüatör kullanılarak kontrol edilen 3D baskılı bir sığınaktan oluşan deney düzeneği kullandık. PLA malzemeden üretilen 3 boyutlu sığınaklar, üç farklı uzunlukta (7 cm, 14 cm ve 21 cm) ve iki yüzey özelliği varyasyonunda (pencereli ve penceresiz) hazırlandı. Sığınak, bilgisayardaki özel bir kod yardımıyla 0,10 Hz ile 2,05 Hz aralığındaki sinüslerin toplamı ile hareket ettirildi. Deney koşulları dört değişkenin manipülasyonunu kapsamıştır: 1) aydınlatma seviyeleri (karanlık, loş ve aydınlık), 2) sığınak yapısı (pencereli veya penceresiz), 3) sığınak uzunluğu (7 cm, 14 cm, 21 cm) ve 4) iletkenlik seviyeleri (düşük, orta ve yüksek). Her tür için (N=5 ayrı balık olmak üzere) toplam 54 farklı duyusal koşul test edildi. Türler arasında istatistiksel olarak anlamlı bir fark vardı; kara hayalet bıçakbalıklarının RMSE değerleri cam bıçakbalıklarına göre daha düşüktür. Sığınak izleme performansı için en önemli parametreler aydınlatma ve uzunluktur. Ayrıca iletkenliğin izleme performansı üzerinde önemli bir etkisi vardı. Ancak pencere durumunun sığınak takip performansı üzerinde aynı etkiyi yaratmadığını belirledik. Ayrıca 'Tür ve Uzunluk', 'Tür ve Pencere', 'Aydınlık ve Uzunluk', 'Aydınlık ve Pencere' koşulları arasında istatistiksel olarak anlamlı etkileşimler bulduk. Sığınağın uzunluğunun, sığınakların pencereli olup olmamasına göre balıkların sığınak takip performansı üzerinde daha büyük bir etkisi olmuştur. Sığınaklarda iyi aydınlatılmış koşullarda açılan pencerelerin varlığı takip performansı üzerinde küçük bir etkiye sahipken, karanlık koşullarda sığınaklarda pencerelerin bulunması takip performansını olumlu etkileyerek sonuçlarda büyük fark yaratmıştır. Sonuçlar sığınak izleme davranışının düşük iletkenlikte çok daha başarılı olduğunu gösterdi. Yüksek iletkenliklerde elektrosensör belirginliği tehlikeye girdiğinde balık görsel bilgileri ağırlıklandırdı.

Keywords: zayıf elektrikli balık, sistem tanılama, duyusal belirginlik, takip davranışı, aktif algılama

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CONTENTS

Page

TABLES

Page

FIGURES

ABBREVIATIONS

3D	:	**T**hree **D**imensional
ABS	:	**A**crylonitrile **B**utadiene **S**tyrene
ANOVA	:	**A**nalysis **o**f **V**ariance
°C	:	**C**entigrade
cm	:	**C**entimeter
dB	:	**D**ecibels
e.g.	:	**F**or **E**xample
fb	:	**F**requency **B**ase
FFT	:	**F**ast **F**ourier **T**ransform
FRF	:	**F**requency **R**esponse **F**unction
g	:	**G**ram
Hz	:	**H**ertz
i	:	**A**n **I**maginary **N**umber **C**alled **I**ota
LED	:	**L**ight-**e**mitting **D**iode
lx	:	**U**nit **o**f **I**llumination
min	:	**M**inute
ms	:	**M**illisecond
N	:	**N**umber
pH	:	**p**otential **o**f **H**ydrogen
PLA	:	**P**olylactic **A**cid
RMSE	:	**R**oot **M**eans **S**quared **E**rrors
ROS	:	**R**obot **O**perating **S**ystem
r(t)	:	**I**nput **S**ignal
sec	:	**S**econd
t	:	**t**ime
y(t)	:	**O**utput **S**ignal

1. INTRODUCTION

1.1. Weakly electric fish as a model organism

The problem of how an animal processes the information it receives from sensory sources while producing a behavior and which information is weighted in the neural mechanisms in the process until it produces a motor output remains an unsolved question for researchers [1, 2].

Advanced and specialized systems, such as electrosensing in weakly electric fish, exemplify their ability to adapt to environmental conditions [3]. These fish serve as an great model organism, providing valuable information to explain the processes underlying sensory acquisition [4–8].

The unique anatomical features of weakly electric fish, including their ability to follow a moving refuge in a smooth and linear axis with their ribbon-like fins extending under their bodies, and their ability to sense their environment with electrosense in addition to visual sense make them a very special model organism for performing behavioral experiments in the laboratory [2, 4]. Weakly electric fish offer several advantages for researchers:

- A unique and highly specialized sensory system based on electrolocation and electrocommunication [9–11]
- A simple well-defined electrical organ composed of specialized cells called electrocytes [12]
- The electrical signals produced are stable and repeatable [3, 10]
- Behavioral flexibility that can be studied in laboratory settings
- It is of interest for neurobiological research because of its simple nervous systems
- Opportunity to work in comparison with different types

- Most importantly their ability to adapt to laboratory conditions

We conducted experimental studies with *Apteronotus albifrons* (Black Ghost Knifefish) and *Eigenmannia virescens* (Glass Knifefish), which are species of gymnotiform wave-type weakly electric fish.

Besides the anatomical similarities, some differences exist for the two types of weakly electric fish. The black ghost knifefish produces electric fields at lower frequencies than the glass knifefish [13]. The Black ghost knifefish, whose natural habitat is mostly turbid waters, has weaker vision so their electrosense is stronger. In glass knifefish, this species, which is a swimmer of clearer waters, has a much sharper vision [14].

1.2. Refuge Tracking Behavior

One of the tools that weakly electric fish use to modulate information from sensory cues is body movements. They can perform tail bending and back-and-forth maneuvers by waving the long ribbon-like fin that extends under their body. Thanks to their innate refuge instinct and unique physical characteristics, they can follow a moving refuge in a smooth linear axis [7, 8, 15]. This pattern of behavior is called ‘refuge tracking behavior’ and is also seen in their natural habitat; they often hide among plant roots, rocks, and suspended objects in streams [4, 16].

Tracking performance of weakly electric fish during refuge-tracking behavior mainly relies on two different sensory modalities: vision and electrosense [4, 16]. These fish can detect both self-generated and external electric fields due to the presence of different sensory receptors [14]. In addition to their sensory systems that bear similarities to eye-like photoreceptor organs, they also have tuberous systems unique to fish. This unique tuberous system gives fish the ability to perceive objects even in the dark. Unlike other living species, they use internally generated electrosensory signals to detect objects in their environment, catch prey, and communicate socially.

A behavior similar to the refuge-tracking of weakly electric fish is also seen in flower-tracking(nectar-feeding) behavior in hummingbirds and hawkmoths. A hawkmoth can cope with the difficult task of sustaining this behavior by hovering in the same position as the flower while feeding on a flower moving in the wind [17]. In addition to their mechanosensory systems located in their antennae, insects also gather crucial information about the flower's motion through the proboscis inserted into the flower's nectar, further contributing to their flight control capabilities. Moreover, while doing this, studies have been conducted on how tracking performance adapts to environmental conditions and how it weights information from visual and mechanosensory organs among species adapted to different light conditions [18].

1.3. Contributions

Our empirical study contributes to the literature in several ways. First, using weakly electric fish in behavioral experiments provides a unique contribution. Previous research showed that the presence of the anal fin under the abdomen of the fish allowed them to exhibit balancing behavior by producing counter-movements while tracking refuge. This anal fin, which produces opposing movements, allowed them to exhibit balancing behavior. It has been shown that the morphological design of weakly electric fish contributes to movement control by acting on neural mechanisms [19].

Second, this study investigated the linearity of multi-sensory integration with simultaneous presentation of visual and electrosensory cues. Consistent with previous studies investigating the control theoretical framework of biological systems, system identification techniques were used to investigate the sensory-motor role of refuge tracking in fish. The extent to which fish modulate their dependence on visual and electrosensory cues under conditions where electrosensory input is compromised, caused by high conductivity levels, was investigated [6].

Moreover, research has shown that active sensing movements of weakly electric fish are regulated by feedback [20, 21]. The magnitude of the sensory shift in light was found to be much less than in the dark. The behavioral outputs of fish were compared with open and closed loop experimental approaches. Thus, in this study, examining how lighting conditions affect the spatial distribution of fish in the refuge aims to present new perspectives on the feedback control mechanism of behavior and reveal the relationships between different categories of active sensing movements and fluctuations in sensory information [8].

Our main question in this study was: How does the refuge tracking performance of fish change under different sensory salience conditions? To address this question we conducted behavioral experiments to observe the effects of different environmental conditions on the tracking performance.

In the previous studies, behavioral experiments in which environmental conditions were manipulated with limited variability involved individuals of one species of weakly electric fish. There was no comprehensive experimental study with individuals of different species. Going beyond the previous studies, we conducted a study in which visual and electrosensory information was weighted according to changing environmental conditions in individuals of two different species of weakly electric fish with gymnotiform wave type.

We can summarize our contributions as follows:

- We designed experiments in different refuge structures by developing the combination of experiments that Uyanık et al. [8] performed in their study in which they determined the categories of active sensing movements of weakly electric fish.
- We investigated the weight of visual and electrosensory cues under different conditions of sensory salience.
- We conducted experiments with two species of weakly electric fish, *Eigenmannia virescens* and *Apteronotus albifrons*, which allowed us to do a comparative analysis.
- We used two different metrics to measure the fish's refugee tracking performance. One of them was RMSE and the other was the tracking error.

- We interpreted the findings we obtained with mixed design five-factor ANOVA(Abbreviation for Analysis of Variance).
- This study provided the groundwork for comparative system identification and mathematical modeling studies to be planned in the future.

1.4. Scope Of the Thesis

Our analysis was based on interspecies performance comparison of the fish's refuge tracking behavior under different sensory salience conditions and interpretation of these effects.

The response of fish to changes in different environmental conditions can be observed to understand how the fish's electrosensory systems work. To this end, we observed fish's behavior by manipulating the environmental conditions, which we assumed that could affect fish's behavior in their natural environment.

For instance, refuge length and spatial heterogeneity can produce dramatic effects in fish electroreceptor organs. Windows in the refuge can cause the electric field strength to vary spatially and cause the fish to weight the visual and electrosensory cues differently.

Moreover, the conductivity of the water in which fish are can affect their electrosensory systems. It is known that the bioelectric signal amplitude decreases as the conductivity increases [22]. Decreased bioelectric signal amplitude may have various effects on the electrosensitivity of weakly electric fish, affecting their ability to navigate and detect objects.

In examining the weight of sensory modalities in refuge-tracking performance, we investigated whether there are intra- and inter-species differences even though different species of weakly electric fish have similar electrosensory systems.

We measured the performances of the South American wave-type gymnotiform species *Apteronotus albifrons* and *Eigenmannia virescens* in a refuge tracking task in which we varied the quality of visual and electrosensory information, through changes in illumination, refuge length, the presence of windows in the refuge structure and conductivity.

Our hypotheses were as follows: 1) We expected similarities in refuge-tracking behavior between the Black Ghost Knifefish and the Glass Knifefish, as they have similar anatomical features. 2) We expected lighting to have the greatest effect on tracking performance, as demonstrated in previous studies. 3) We expected the length of the refuge to have an effect on the fish's perception of position, to be reflected in the results. 4) We expected that the windows to be opened on the refuge walls would affect the electrosense of the fish, influencing the tracking behavior. 5) We thought that when the conductivity of the experimental tank water was changed from low to high, the tracking performance of the fish would be affected by this situation.

The aims of this thesis can be listed as follows:

- Comparative analysis of the refuge tracking performances of two species of weakly electric fish under different sensory salience conditions (conductivity, illumination, windows, length). There is no such study in the literature before.
- The results of refuge tracking performance among different species of weakly electric fish will form the basis for future comparative system identification work.

1.5. Organization

The organization of the thesis is as follows:

- Chapter 1 presents the scope of our thesis and what we aim with the experimental study
- Chapter 2 provides detailed information on weakly electric fish and background information on their refuge-tracking behavior and presents the review of the studies in the literature
- Chapter 3 demonstrates how we performed the experiments with the experimental setups we designed, the housing of weakly electric fish in the laboratory environment, the mechanical architecture of the experimental setup, the image processing process from the experimental data, and the experimental procedure

- Chapter 4 shows our experimental results and detailed analyzes of these results in the discussion section
- Chapter 5 outlines the thesis and possible future directions

2. BACKGROUND AND RELATED WORKS

2.1. Weakly Electric Fishes

Trying to understand the mechanism of neural processes in creatures with much simpler nervous systems than humans can provide valuable information about the functioning of our own complex brain. Weakly electric fish serve as an invaluable model organism for elucidating the processes underlying sensory acquisition [5].

Weakly electric fishes, inhabitants of freshwater environments, exhibit a functional 'phylogenetic' division into two branches: 1) Gymnotiformes found in South America and 2) Mormyriformes found in Africa [23]. Electric organ discharges in these fish can be classified as 'wave' or 'pulse' types according to the voltage waveforms measured between the electrodes near the head and tail [24, 25]. In our experimental studies, we included *Apteronotus albifrons* belonging to the Apteronotidae family and *Eigenmannia virescens* species belonging to the Sternopygidae family as pulse-type gymnotiform specimens.

Weakly electric fish use internally generated electrosensory signals to sense objects in their environment, catch their prey, and engage in social communication [26]. Movement is facilitated by the generation of propagating waves using highly agile ribbon-like anal fins. Due to different sensory receptors, weakly electric fish can perceive both self-generated and external electric fields [27].

The electrical organs responsible for generating electrical signals of varying strength and duration for defense, aggression, communication, and electrolocation consist of electrically excitable cells called electrocytes [28]. These electrocytes are controlled by groups of neurons in the brain known as pacemakers or command nuclei that establish the basic rhythms of electrical organ discharges [29]. Higher brain centers change these rhythms to create different electrical behaviors.

Weakly electric fish possess a dual-electrode sensor system comprising the ampullar and tuberous systems. The ampullar system facilitates the perception of external stimuli while the tuberous system enables the fish to perceive its own electric signals. Although the ampullar system shares similarities with photoreceptor organs found in humans, such as the eye, the tuberous system is exclusive to fish. This unique tuberous system gives fish the ability to see in the dark. If humans possessed an analogous system, it would offer enhanced vision during nighttime conditions [30].

Fish of the species *Apteronotus albifrons* are entirely black except for two white rings on the tail and, a white stripe that can sometimes extend down the back as a stripe, and a flame-like spot on the snout. That's why it's called **'Black Ghost Knifefish'**. By waving the long fin that extends under their body, they can make tail bending and forward-backward maneuvering movements. These fish engage in active hunting primarily during nocturnal periods, where active electrosense is hypothesized to serve as a crucial mechanism for prey localization and capture. This hypothesis is substantiated by the fish's proficient ability to catch prey even in the absence of visual sensory input [14, 31]. These nocturnal fish have developed the ability to generate and sense weakly electric fields called electrogenesis and electrosensation. This electric field produced by a special electric organ is called as the electric organ discharge (EOD). In their natural environment, these fish use self-generated electrical organ discharges (EODs) for sensory processes such as navigation and communication.

Eigenmannia virescens, one of the fish in the Sternopygidae family, is characterized as the **'Glass Knifefish'**. These fish, whose natural habitats are Panama, freshwater rivers in South America, and the Amazon basin, have translucent body anatomy [14].

Active electrolocation is thought to play an essential role in the ability of weakly electric fishes to detect and capture prey. This assumption is supported by successful prey captures in the absence of visual cues, the predominance of peripheral receptors, and significant brain tissue dedicated to high-frequency electroreceptors. For example, the adult black ghost knifefish has approximately 15.000 tuberous receptor organs distributed over the body surface, approximately 700 ampullary receptor organs, and 300 neuromasts for mechanosensory lateral line perception. black ghost knifefish generally produces electric fields at lower frequencies than glass knifefish, which emits continuous, almost sinusoidal electric fields between 200 and 700 Hz [13].

The accessibility and availability of weakly electric fish further distinguish them as an ideal choice for laboratory studies. In addition, their utilization in research offers notable cost-effectiveness and simplicity. These attributes grant these fish a unique advantage and provide researchers with exceptional opportunities for investigation. Previous studies in the literature have demonstrated the remarkable forward and backward maneuverability of weakly electric fish, primarily attributed to the elongated anal fin extending along their bodies [7, 32]. Moreover, their inherent refuge instincts and specific physical characteristics enable them to exhibit smooth linearity in their refuge-tracking behavior [7, 8, 15].

In conclusion, gymnotiform fish, particularly black ghost knifefish and glass knifefish, are valuable models for studying behavioral plasticity and the mechanisms involved in generating and sensing weakly electric fields. Their electric organ discharges play essential roles in various sensory processes, including prey detection and communication, making them intriguing subjects for further research on electroreception and sensory integration in vertebrates.

2.2. Refuge Tracking Behavior

Weakly electric fish take refuge in tree trunks, plant roots and rock fragments, which they instinctively find in rivers and streams in their natural environment. Thanks to their highly maneuverable ribbon-like anal fins that run along their body, they exhibit a remarkable ability to track a dynamically controlled mobile refuge. They demonstrate a remarkable ability to move both forward and backward within the refuge, maintaining their distance from the moving refuge. This distinctive behavior is often described as the refuge-tracking behavior [4, 16].

The Figure in 2.1 shows a graph of the refuge frequency signal and the tracking responses of the fish for a 60 second test trial in the time domain. The red line represents the refuge, the blue line represents the fish.

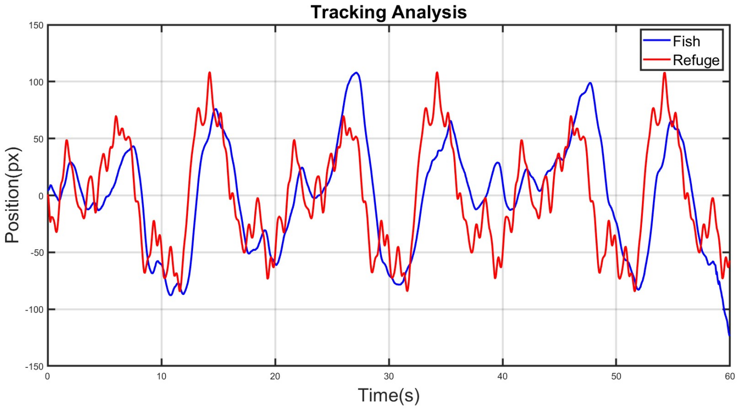


Figure 2.1 An example of graphical representation of Refuge-Tracking Behavior of Weakly Electric Fish in time domain

Fish rely on two sensory perceptions to perform this smooth tracking behavior: vision and electrosense [6, 16]. While it shows better tracking behavior in the presence of visual information, it appears to exhibit other behaviors that may improve sensing.

Weakly electric fish generate an oscillating electric field through the electric organ that serves prey-catching and social communication behaviors [2, 3]. While inside the refuge, it measures fluctuations through voltage-sensitive sensors on its skin to create an electrosensory image of the refuge. Electroreceptors are concentrated in the head region, but scattered throughout the body [33]. In the refuge, fish can swim forwards and backwards without changing direction [4, 7] by regulating visual and electrosensory information, moving its line-like anal fin, and slightly bending its body. Considering similar studies in the literature [4, 8, 15, 17], fish successfully followed a refuge that was moved in a sinusoidal (predictable) or sum of sinuses (unpredictable) [7].

In our study, we performed a similar experiment with 3D-printed refuges. The refuges shown in Figure 2.2 were moved using a stimulus at total frequencies in the 0.10 Hz to 2.05 Hz frequency range using a special code on the computer. In particular, frequencies that are prime multiples of a fundamental frequency $f_b = 0.05$ Hz were chosen to reduce the confounding effects that can be caused by harmonic phenomena. The top chart represents 5 different experimental trials under each experimental condition, while the chart below shows mean fish and mean refuge data.

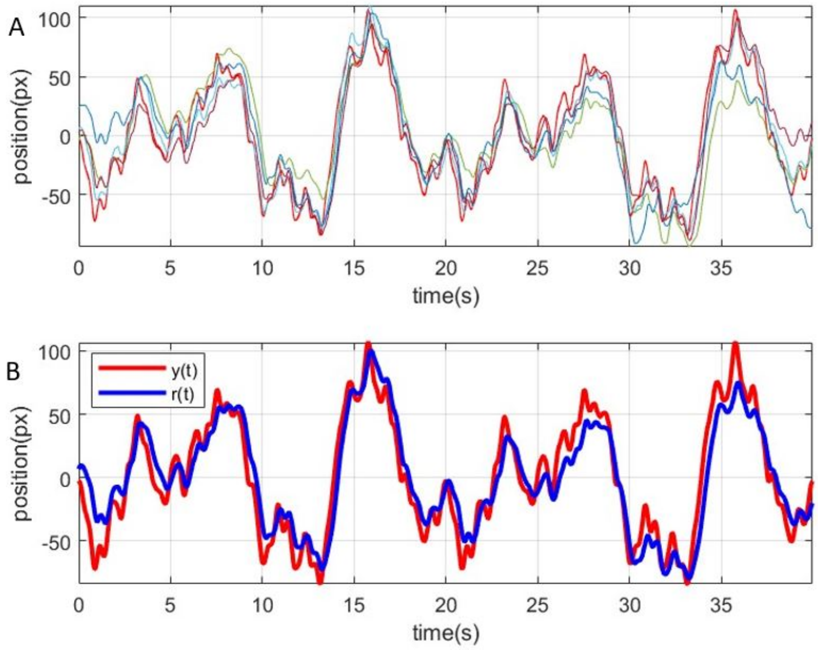


Figure 2.2 Graphical representation of refuge-tracking behaviour of black ghost knifefish in time domain. A displays 5 trials in one condition, while B displays average fish and average refuge data.

2.3. Previous Works

2.3.1. Active sensing motions in weakly electric fish

Stamper et al. [15] demonstrated that weakly electric fish derive advantages from the integration of their visual and electrosensory systems in regulating locomotor behavior. While the benefits of visual and electrosensory information for fish were examined, research also investigated the energetically costly movements exhibited by fish in the absence of visual cues.

Their study revealed a correspondence in tracking performance with previous investigations [4, 7]. In the absence of light, the fish engaged in active sensing movements, resulting in increased locomotion energy costs. To investigate the impact of body oscillations on electrosensory processing, experiments were conducted by varying water conductivity levels. The results revealed that changes in illumination and conductivity influenced the tracking performance. It was observed that the fish exhibited heightened activity in the dark, which influenced electrosensory feedback. Additionally, the tail-twisting movements of the fish contributed to sensory processing. Overall, the study demonstrated the utilization of active sensing movements in electrosensory feedback, thereby shaping spatiotemporal information.

2.3.2. Opposing forces that contribute to stability and maneuverability

Sefati et al. [19] conducted a study on glass knifefish, a weakly electric fish species, to explore the relationship between antagonistic forces and locomotor control. In this species, characterized as a glass knifefish, the thrust force is generated by the undulating movements of a long anal fin. The presence of this anal fin beneath their abdomen enables weakly electric fish to exhibit stabilizing behavior during refuge tracking through the production of opposing movements. The experimental findings and a mathematical model derived from fish swimming dynamics [34] revealed that generating opposing forces is a strategy that enhances maneuverability and maintains balance. Additionally, it had been suggested that this morphological design observed in weakly electric fish would not only contribute to locomotion control but also have implications for neural mechanisms.

2.3.3. Adjusting amplification of visual and electrosensory inputs to regulate locomotor coordination

Researchers have conducted experiments involving weakly electric fish to investigate the linearity of multisensory interaction [6, 35]. Specifically, they examined the simultaneous presentation of visual and electrosensory cues that were either contradictory or consistent. The study focused on understanding how the fish would weigh sensory information about the disparity between visual and electrosensory cues. The sensorimotor control process can be conceptualized as a closed loop, whereby perception influences behavior, and subsequent changes in the environment are perceived and further shape the process. Control theory offers a framework for understanding animal behavior, encompassing the translation of sensory inputs to motor outputs. Closed-loop neuromechanical modeling has been employed to implement feedback control in various biological systems, such as the tracking of flowers by moths [36] and the tracking of refuges by weakly electric fish [8]. In line with previous studies investigating the control theoretical framework of biological systems, Sutton et al. [6] employed system identification techniques to investigate the sensorimotor task of refuge tracking in fish. By leveraging the inherent refuge-tracking behavior of fish in confined spaces within their natural habitat, Sutton et al. [6] successfully quantified the dynamic alterations in the relative weighting assigned to visual and electrosensory information during a sophisticated locomotor task. By implementing a custom-built experimental configuration, the researchers were able to investigate the extent to which fish modulate their reliance on visual and electrosensory cues under conditions of compromised electrosensory input caused by elevated conductivity levels. Particularly, the results revealed that fish greatly emphasized visual information when confronted with compromised electrosensory data due to high conductivity levels.

2.3.4. Movement categories for refuge tracking of weakly electric fish

Glass knifefish exhibits regulatory control over its locomotion when faced with alterations in sensory cues within a refuge. The behavior of tracking a refuge can be categorized into two fundamental components: smooth tracking behavior and active sensing movements. Theoretically, these fish can optimize their movements while tracking refuges that vary in length and heterogeneity. They consistently maintain their position relative to the refuge by employing strategies such as extending their head in front of the refuge and bending their tail, thereby avoiding departure from the refuge and engaging in back-and-forth movements.

In their investigation, Uyanık et al. [8] examined the effect of illumination conditions on the positioning of glass knifefish within a refuge. The spatial distribution of fish within the refuge demonstrated a uniform pattern, characterized by a significant occurrence of fish extending their heads outside the refuge. This phenomenon was attributed to the fish's tendency to display increased levels of non linear motion in dark conditions.

Furthermore, a transition from light to dark conditions was observed to influence the tracking behavior negatively. The study aimed to offer novel insights into the feedback control mechanism of animal behavior by examining the relationships between distinct categories of active perception movements exhibited by glass knifefish and the fluctuations in sensory information.

2.3.5. System identification approach for modeling brightness dependent adaptations in flower tracking

Researchers have drawn from systems identification approaches in flight control in flies and hawkmoths, in balanced body movement in humans, and in refuge-tracking of weakly electric fish [37–41]. Insects possess remarkable coordination and visual body mechanics that enable them to navigate obstacles and approach flowers during flight control with great precision [42]. They skillfully insert their proboscis into the flower's nectar to extract nutrients [43], and adeptly track the flower's movements in response to wind disturbances [17, 44], allowing them to forage for food both day and night. In addition to their mechanosensory systems located in their antennae, insects also gather crucial information about the flower's motion through the proboscis inserted into the flower's nectar, further contributing to their flight control capabilities.

When investigating the variations and responsiveness of flower tracking behavior in insects with comparable anatomical structures and flight strategies [45], careful consideration was given to the eye anatomy responsible for receiving visual information within similar acceptance angles. Moreover, the analysis encompassed the photoreceptor systems that specifically and respond to flower motion, and the central nervous system's integral role in integrating and assigning weights to these diverse sensory inputs.

Sponberg et al. [17] employed a systems identification methodology to elucidate the alterations in flower tracking behavior with varying light intensities in a crepuscular hawkmoth species. They examined the feedback nature of sensorimotor processing and the dynamic patterns that influence behavior. Building upon this investigation, Stockl et al. [18]

conducted a comparative study involving three distinct hawkmoth species to explore this phenomenon further.

Prior research has demonstrated the linearity and time-invariance of flower tracking behavior [17, 44]. Consequently, the frequency response of the system, quantified by a complex number, can be characterized by two fundamental aspects: the magnitude, representing the gain, and the angle, denoting the phase difference.

Across all light conditions, behavioral adaptations were observed in all hawkmoth species. There were no significant disparities observed in tracking performance at distinct frequency points. Specifically, when comparing the flower tracking performance statistically between species, no significant distinction was found between the characteristic frequencies of *Manduca sexta* and *Deilephila elpenor*. It is postulated that the two species, exhibit comparable characteristics in contrast to the diurnal species.

When employing a model to elucidate variations within and between species, it was observed that adaptations to distinct light intensities within a species could be accounted for by incorporating a straightforward delay term. However, delay term alone proved inadequate to explicate the disparities observed between species. Consequently, a scaling factor was included to account for the differences observed among the species adequately.

2.3.6. Examining the effects of changes in mechanisms affecting movement

Despite the varying anatomical features among animals, their behavioral performances exhibit similarities. Uyanik et al. [46, 47] conducted studies to examine how weakly electric fish with distinct morphological characteristics can accommodate their morphological differences. They investigated this by measuring the system responses during the execution of the refuge-tracking task. The authors postulated that animals possess a flexible nervous system that governs body movements, continuously influenced by sensory feedback. This observation brings attention to the factors involved in robust motion control, including implications for the design of robotic engineering systems.

In previous studies, a feedback control model was designed to examine the hypothesis regarding the influential role of the central nervous system in exerting strong feedback control mechanisms. These studies, conducted by Cowan and Fortune [4] and Roth et al. [7], introduced a computational framework enabling modifications to the controller-plant relationship. The modeling results provided evidence that feedback-based task control facilitated the adaptation of animals to inter-individual variability. To comprehend the relationship and impact of closed-loop control, it is imperative to investigate the interplay between behavioral performance and neural mechanisms involved in animals. Previous studies [18, 48] have used a control theoretic approach to model tracking behavior under different salience conditions.

In this study our aim was to investigate the effects of perceptual salience on the refuge tracking performance of two different weakly electric fish species based on studies in the literature.

3. MATERIAL AND METHODS

3.1. Housing of Fish in the Laboratory

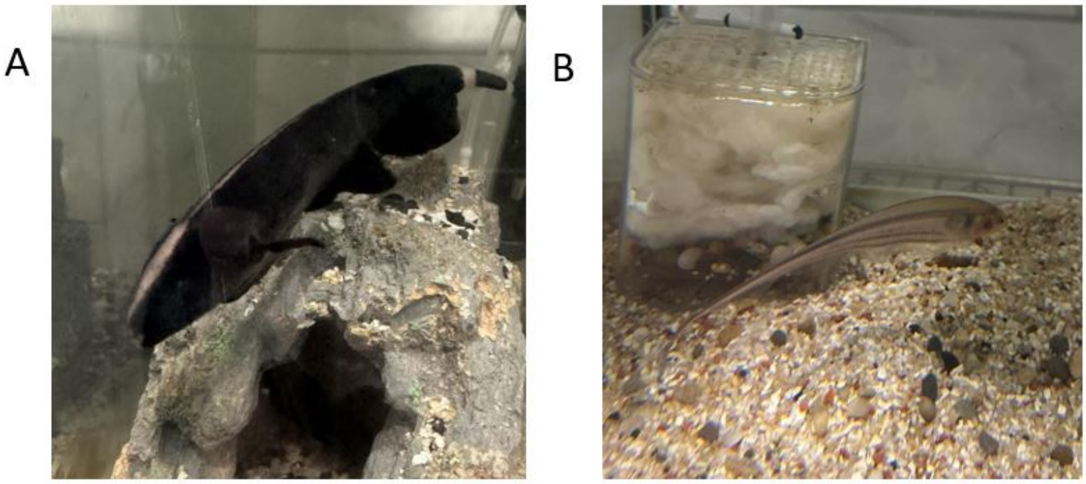


Figure 3.1 For our behavioral experiments, as an example of the species we host in the laboratory, a black ghost knifefish species is displayed in A and a glass knifefish species is displayed in B.

Weakly electric fishes depicted in Figure 3.1 are captured from their natural habitat and subsequently transferred to laboratory environments due to the absence of commercial breeding programs for these species. We hosted them in the laboratory based on previously published guides [2].

The initial health of fish is closely linked to the conditions prevailing in their capture environment. Factors such as parasite burden and transport conditions play a critical role in determining the overall well-being of the fish. During the initial weeks following their arrival in the laboratory, particular attention is given to ensure optimal conditions, as this period is highly crucial and often associated with a significant loss of specimens. Therefore, strict quarantine protocols are implemented during this early phase, with strict adherence to hygiene measures to prevent cross-contamination with other aquariums. Maintaining an appropriate water temperature between 24 °C and 26 °C and ensuring an ample oxygen supply in the aquarium tanks are essential for promoting their health.

Glass knifefish, being a highly social species, is commonly maintained in laboratory settings alongside conspecifics. In contrast, due to its aggressive behavior towards individuals of the same species, black ghost knifefish is housed individually in aquariums [13, 49, 50].

Weakly electric fishes easily eat frozen bloodworms, frozen or live shrimp, and live land worms. Feeding regimens for these fish may involve infrequent intervals, such as every other day or every 3 days, to prevent overfeeding and potential water pollution caused by food residues. Given their natural habitat near the equator, a recommended day-night cycle of 12 hours of light and 12 hours of darkness is suitable for these fish. Maintaining optimal water parameters is crucial, including a pH range of 6.5 to 7.5, water temperature between 24 °C and 26 °C, and water conductivity within the range of 25 to 2000 μS.

3.2. Mechanical Architecture

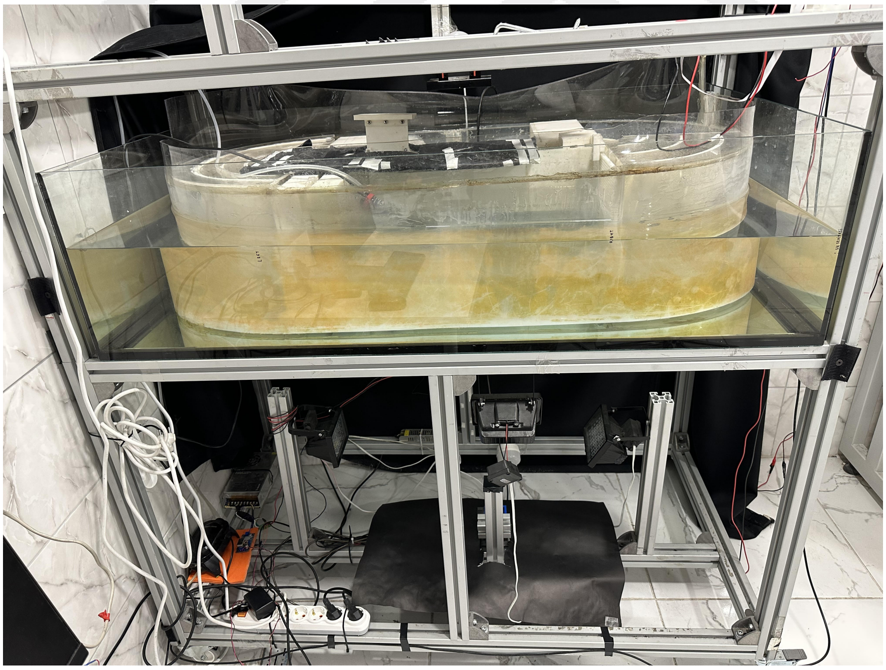


Figure 3.2 The figure shows the design and architecture of the experimental tank in which we performed the behavioral experiments.

The experimental configuration depicted in Figure 3.2 comprised a specialized aquarium system designed to facilitate the execution of the tracking behavior by the fish within a mobile PLA tube. The movements of the PLA tube were facilitated by a linear motor, characterized by its single-axis motion capability and high precision. To replicate a natural environment, the aquarium was configured as a river tunnel, utilizing a water pump connected at both ends to establish continuous water circulation. Within the experimental area, honeycomb perforated filters were strategically positioned at the water inlet points of the aquarium to maintain a consistent water flow.

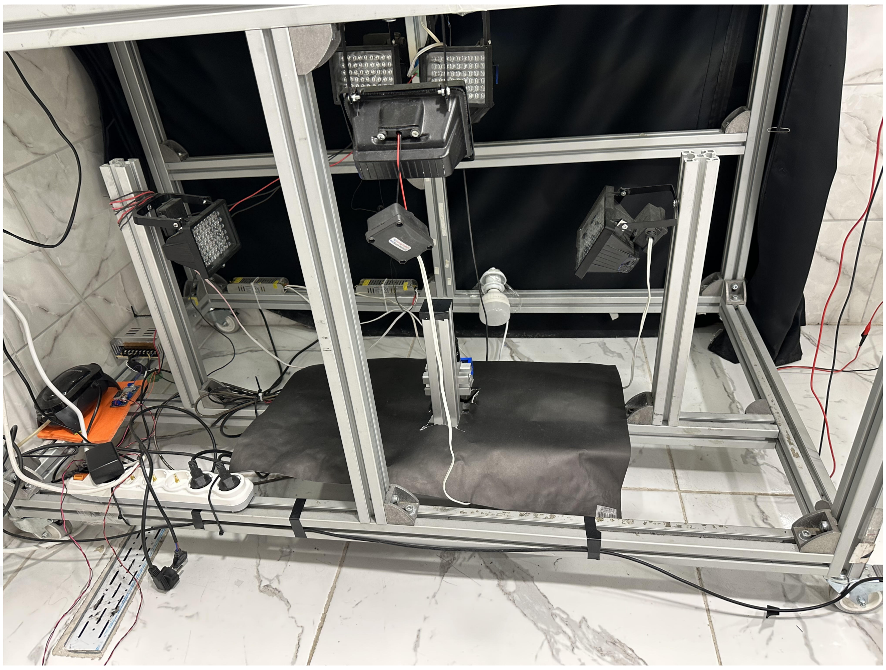


Figure 3.3 The figure shows the section below the experimental area where the camera is placed at a right angle.

The camera was positioned at a right angle to provide a direct view under the refuge. As shown in Figure 3.3, it required the removal of the bottom of the refuge to enable unobstructed camera recording. Thus, it provided a comfortable view of the refuge and the silhouette of the fish through the clear glass.

The camera recorded the behavioral responses of the fish, denoted as *y(t)*, in relation to the movements of the refuge, denoted as *r(t)*. Employing a customized MATLAB code, the fish and refuge locations were derived from the resulting video footage.

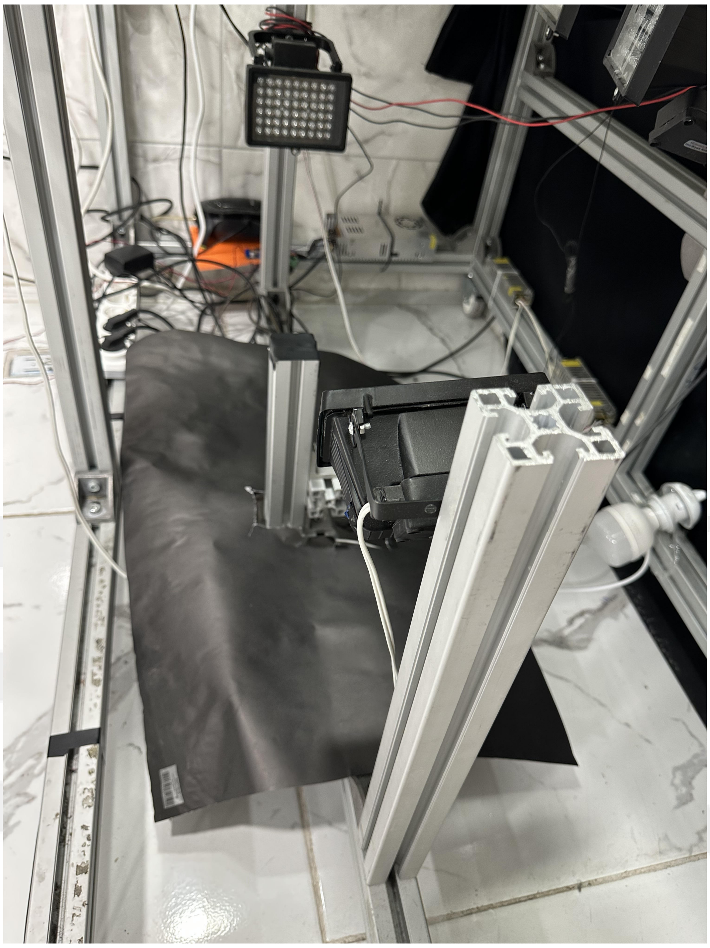


Figure 3.4 The figure shows the location of the camera and infrared lights under the experimental tank.

In order to facilitate the visibility and detection of fish in the experimental area in dimlight and dark experiments, infrared light sources were placed at the bottom of the test tank in different directions.

Additionally, LED bulbs were placed to allow the fish to rest between experiments. These were opened during 1-minute rest breaks. Although infrared lights were used in addition to ambient light in light illumination experiments, only infrared lights were used in dark experiments.

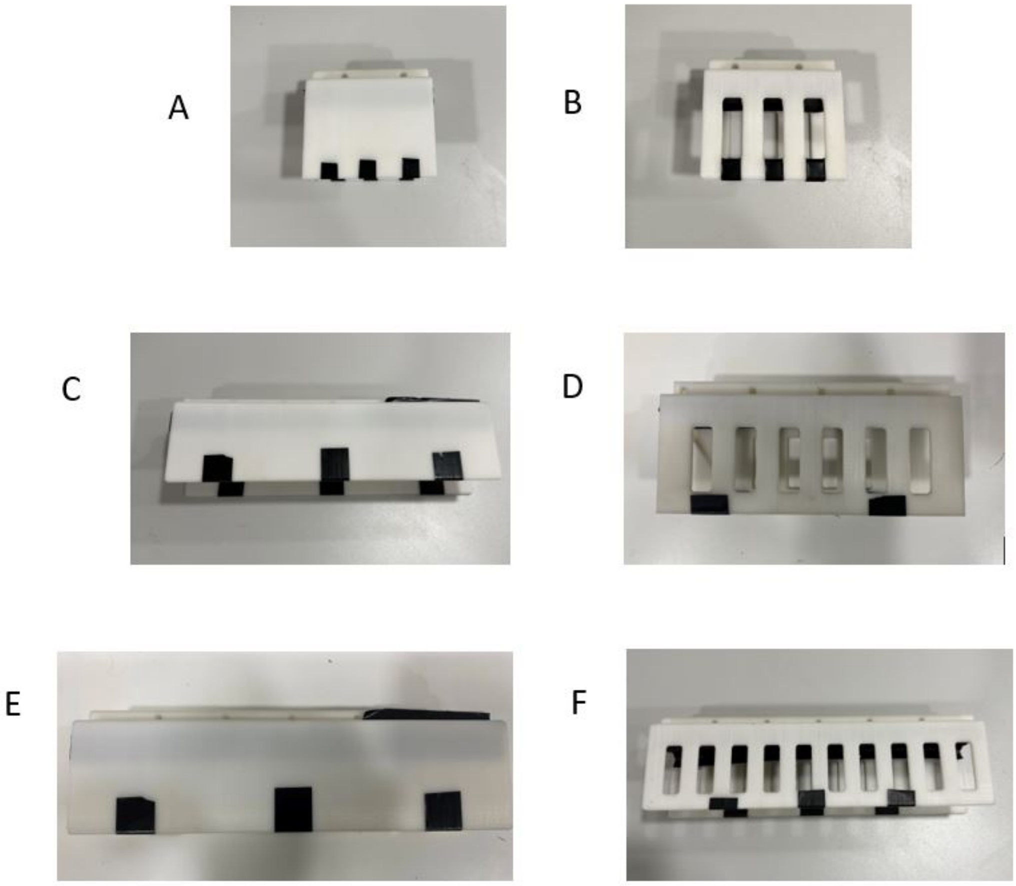


Figure 3.5 Refuge structure figure shows the 7 cm windowless refuge in A, the 7 cm window refuge in B, the 14 cm windowless refuge in C, the 14 cm window refuge in D, the 21 cm windowless refuge in E, and the 21 cm window refuge in F.

Differently constructed refuges shown in Figure 3.5 were used to provide hiding places for the fish and to facilitate the observation of their behavior. Windows were placed in PLA tubes to increase sensory salience under different experimental conditions [8].

Variations in refuge lengths and the presence or absence of windows have a substantial influence on neural mechanisms. The distribution of windows yields pronounced effects on the electric field detected by the receptors, consequently impacting the tracking behavior of the fish.

Similar to previous investigations, adopting a symmetrical pattern in the design of windowed refuges reduces potential behavioral disparities stemming from spatial heterogeneity. The inclusion of windows in refuges augments the visual electrosensory information available compared to windowless refuges [8].

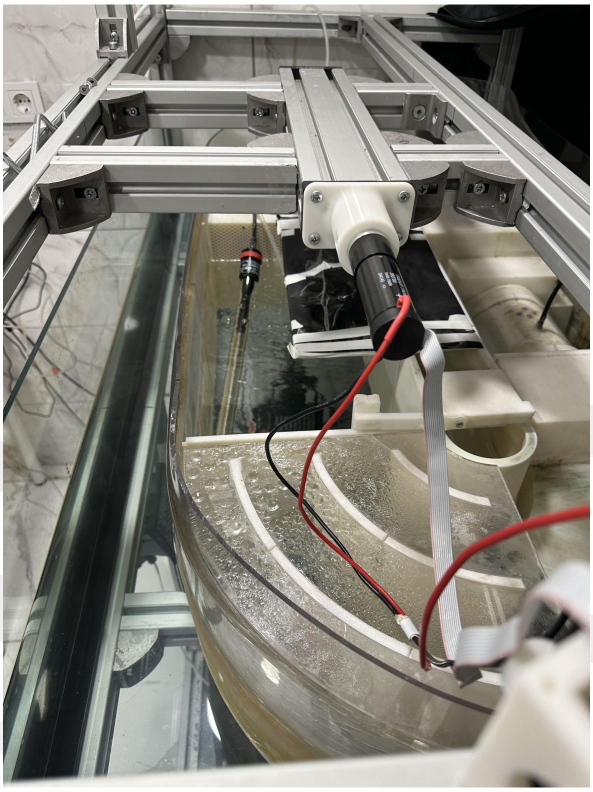


Figure 3.6 The figure shows the part of the experimental setup that enables the movement of the refuge with the help of a computer-controlled linear motor.

One of the fundamental requirements for the experimental setup's electronics and software architecture was the system's real-time operation at high frequencies. To ensure accurate and synchronous measurements, the experimental setup incorporated a real-time electronic and software infrastructure. Consequently, the system's overall architecture was designed to operate on the Robot Operating System (ROS), facilitating the real-time processing and control of data.

The primary software components of the system were executed on the Nvidia Jetson NX card utilizing the Robot Operating System (ROS). The Nvidia Jetson NX card performed key functions, including: (1) initiating and controlling the experimental sequence as instructed by the mainframe, (2) transmitting motion commands to the motor driver and processing feedback regarding the motor position, and (3) acquiring fish positions and raw image frames through bidirectional communication with the secondary board employed for image processing.

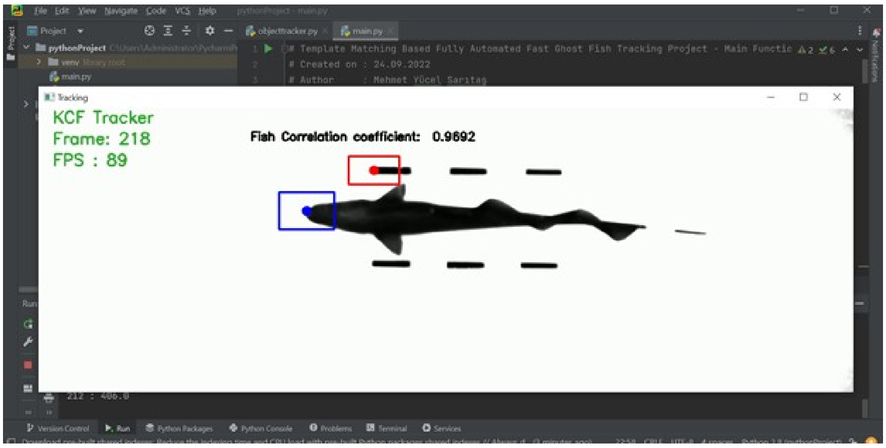
The linear motor's motion control was achieved through a motor driver, operated via the Jetson NX card. Our objective was to enable precise single-axis motion control of the PLA tube with millimeter accuracy. The motor driver transmitted the measured motor positions to the Jetson NX card. To this end, the Jetson NX card triggered the camera periodically at intervals of 0.04 seconds, capturing an image frame. The position data derived from fish detection within the image frame was then transmitted to the Jetson NX . Additionally, a duplicate of the resulting image frame was transmitted over ethernet to the Jetson NX card and subsequently to the host system for recording purposes. Adhering to ethical guidelines, measures were taken to minimize the need for live animal experiments and prevent data loss. Consequently, this data was promptly backed up on a network-attached storage system.

3.3. Image Processing

One crucial component of the data collection system involved accurately determining the fish's position. Fortunately, the absence of objects other than the fish and PLA tube refuge within the experimental area simplified the image processing procedure.

Black ghost knifefish species exhibited a distinct coloration with a black body, a stripe extending down their back, and two white rings on their tail. Consequently, the image processing algorithms were adjusted to account for the specific color contrast associated with these fish. Conversely, glass knifefish were presented in white against a darkened background, allowing for appropriate color contrast adjustments in image processing.

In our study, we employed deep learning algorithms based on artificial neural networks to detect the position of fish in image frames. The Jetson NX card, an integral part of our electronic system architecture, was chosen as a specialized hardware capable of achieving high-speed implementation of this deep learning algorithm. The operational procedure involved initially manually marking a specific region on the fish's head in different image frames. Subsequently, the relevant artificial neural networks were trained, enabling the



```
# Template Matching Based Fully Automated Fast Ghost Fish Tracking Project - Main Functic
# Created on : 24.09.2022
# Author      : Mehmet Yücel Saritas
```

```
KCF Tracker
Frame: 218
FPS : 89
Fish Correlation coefficient:  0.9692
```

Figure 3.7 The figure displays a frame from the image processing process with 'Python' software, which has been specially developed for fish and refuge marking.

program to automatically identify the fish's position in other image frames based on the learned network structure.

In addition, we also used target tracking software called 'DeepLabCut', an open source tool for fish and refuge marking. The process shown in Figure 3.7 involved selecting a square area corresponding to the fish's head to ensure that each trial was included in the program and accurate tracking data was extracted. The program then generated an output in the form of an Excel file containing the locations of both the refuge and the fish.

3.4. Experiment Procedure

All experimental procedures in this study were conducted in accordance with the ethical guidelines and regulations set forth by the Hacettepe University Ethics Committee. The housing of the fish during the experimental period adhered to established guidelines as outlined in previous studies [2].

Studies examining the influence of sensory salience on the behavioral performance of weakly electric fishes were limited in the existing literature [8, 15]. These studies employed a limited number of constant frequency input signals, resulting in a small data set and challenges for accurate system identification. Conducting separate experiments with fixed frequency signals could lead to fish anticipating the stimulus and minimizing behavioral time delays.

Additionally, performing individual experiments at each frequency could considerably increase the already substantial number of required experiments. To overcome these limitations, we employed a complex stimulus signal composed of a sum of fixed-frequency signals, thus addressing these concerns.

Adult specimens of glass knifefish and black ghost knifefish were procured from commercial suppliers and subsequently housed in separate aquariums following a quarantine period. The experimental design encompassed 3-5 replicates conducted under 54 distinct environmental conditions, involving five individuals from each fish species. The species included in the study were the weakly electric fish black ghost knifefish and glass knifefish.

Initially, an experimental schedule was devised, including daily fish feeding with frozen blackworms. During the designated fish feeding period, program settings on the computer were configured. The illumination conditions were adjusted using a luxmeter, ensuring optimal lighting conditions. Furthermore, the temperature of the test tank was measured and maintained within the range of 24°C to 26°C, which is considered suitable for the well-being of the fish. Subsequently, the fish were introduced into the experimental tank and allowed a period of acclimation. Adequate time was provided under appropriate lighting conditions to allow the fish to enter the refuge voluntarily.

All experimental trials involving the two fish species were conducted over 3 to 6 months. Each trial lasted 60 seconds, during which the fish were allowed to track the refuge. Concurrently, video image frames were instantaneously recorded from the motor drive to the computer. To provide intervals of rest and recovery for the fish, the lights were turned on for approximately 1 minute between each experiment. In cases where the fish did not enter the refuge for an extended period, they were gently guided using fishing nets. Additionally, scheduled resting periods were implemented when the fish did not participate in experiments for prolonged periods or failed to enter the refuge even under light illumination conditions. Due to the highly agile movements of glass knifefish species, these fish were temporarily placed in their aquariums during resting intervals. The experimental trials encompassed three distinct levels of conductivity, starting with low conductivity, followed by medium, and concluding with high conductivity. After each fish experiment, the tank water was completely replaced with deionized water to ensure consistency.

Conductivity Formula
50 g calcium sulfate
10 g magnesium sulfate heptahydrate
4 g potassium chloride
1.1 g sodium phosphate

Table 3.1 The conductivity formula to be added to the test tank while conducting conductivity changes was obtained by mixing the chemicals in 1 liter of deionized water at the specified rates.

The materials necessary for adjusting conductivity were accurately weighed using precision scales. Prior to each test, a preparation process was conducted, where the appropriate conductivity level was achieved by gradually introducing the required chemical mixture into the test water within the specified microsiemens (µS) range. Subsequently, the water motor was activated to ensure a homogeneous distribution of the chemical mixture within the water. Prior to each experiment, conductivity adjustment and temperature measurement were performed, along with the measurement of light intensity using a luxmeter. These data were recorded in Excel files using a template specially designed for the experiments.

Experiments covering 54 different environmental conditions which is shown above in the table 3.2 were performed for each of 5 individual fish from both the black ghost knifefish and glass knifefish fishes species:

Illumination	**Windows**	**Length**	**Conductivity**
dark	with window	7 cm	low (less than 50 µS)
dimlight (20-50 lx)	without window	14 cm	medium (about 300 µS)
light (300-500 lx)		21 cm	high (about 700 µS)

Table 3.2 Behavioral experiments were performed in 54 different combinations of manipulation of 4 different variables (illumination, conductivity, windows, length) to measure refuge tracking performance.

We conducted a total of 2700 experiments, on average, involving two fish species. The fishes used in the experiments were individually named and housed in separate aquariums. Black ghost knifefish were identified by the names **İstanbul, Ankara, İzmir, Antalya**, and **Mersin**, while the glass knifefish fish were identified as **Amasra, Ardahan, Gaziantep, Erzincan**, and **Samsun**, representing city names in Türkiye.

3.5. Frequency Response Function

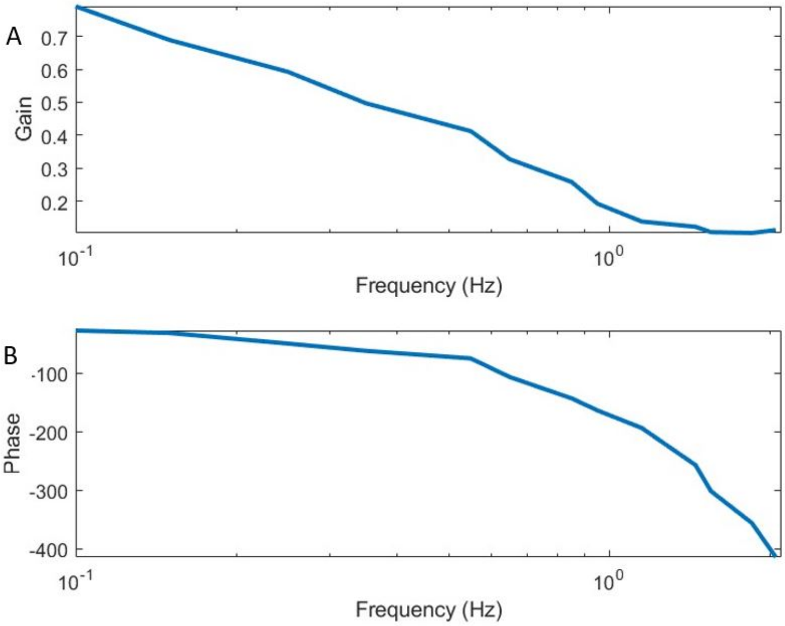


Figure 3.8 Frequency response function features a logarithmic scale on the horizontal axis representing frequency, while the vertical axes represent the amplitude(A) in decibels (dB) and the phase(B) angle in degrees, respectively.

The frequency response function in the Figure 3.8 depicts the frequency response characteristics of a linear system, displaying the relationship between input and output frequencies [7]. It is a metric for assessing performance across various conditions in all experimental trials, enabling comparative analysis of different sensory conditions [15].

The frequencies selected for this section are derived from a base frequency, $f_b = 0.05$ Hz, and were chosen as prime multiples: 2, 3, 5, 7, 11, 13, 17, 19, 23, 29, 31, 37, 41. The corresponding fish movements to these stimulus signals were captured by tracking a specific point on the fish's head using camera images. Given the fish's ability to move freely in three dimensions within the aquarium, our study specifically focused on capturing the fish's one-dimensional movements along the oscillation axis of the PLA tube. The fish movements were digitized from the recorded images using computational methods to obtain this data.

The Discrete Fourier Transform (DFT) is employed to analyze a periodic signal in the time domain, revealing its frequency content and representation. The Frequency response function illustrates the system's response to varying frequencies, presenting separate plots for amplitude and phase [6, 7]. The ideal tracking scenario is denoted by point 1+0i, where the circle intersects the positive axis. By measuring the magnitude of the error signal, which is the distance between this perfect tracking point and the observed transfer function, the fidelity of tracking can be evaluated. Fast Fourier Transform (FFT) is implemented on both the input and output signals using custom MATLAB commands. The output-to-input ratios are assessed for each single-sine trajectory, allowing for the calculation of gain and phase. This analysis provides insights into the system's frequency response characteristics.

3.6. The Calculation of RMSE and Tracking Error

We further computed the Root Mean Square Error (RMSE) values to quantify the disparity between fish movement and refuge movement across different sensory salience conditions.

The RMSE (Root Mean Square Error) quantifies the average magnitude of disparities between fish movement and refuge movement. The RMSE value serves as a measure of the overall deviation between the observed and predicted data. A lower RMSE value signifies a more favorable model fit or greater proximity to the data, indicating a reduced mean error. Conversely, a higher RMSE value indicates an increased average error, diminished accuracy, or inferior model performance.

Here's RMSE calculation:

$$\mathbf{RMSE} = \sqrt{\frac{\sum_{i=1}^{N}(y(t)_i - r(t)_i)^2}{N}}$$

In this formulation, the number *N* represents the number of positions of the average fish and average refuge data in each of the 54 different conditions kept in the Excel files we obtained after the image processing process.

Employing a customized MATLAB code, the fish and refuge locations were derived from the resulting video footage. While r(t) represents the movements of the refuge; y(t) represents the behavioral responses of the fish [51].

The tracking error, represented as ϵ, is defined as the difference in the complex plane between how a fish's frequency response *H(s)* behaves and the perfect tracking conditions, which means the fish's gain should be one and its phase should be zero [18].

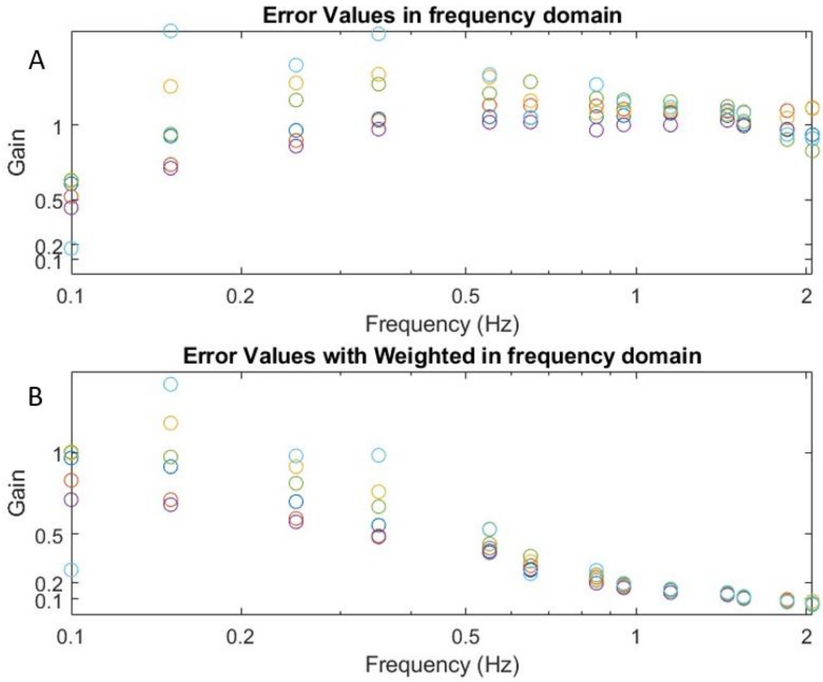


Figure 3.9 While the tracking error, measured as the distance between ideal tracking conditions and the fish's tracking response, is not weighted by frequency values in A, it is weighted by frequency values in B to reflect the behavior of the system much better.

Perfect tracking action is represented by 1+0i in the complex plane and describes the frequency response where the amplitude of the signal is the same as the stimulus and there is no phase delay or time delay.

3.7. Refuge Tracking Performance

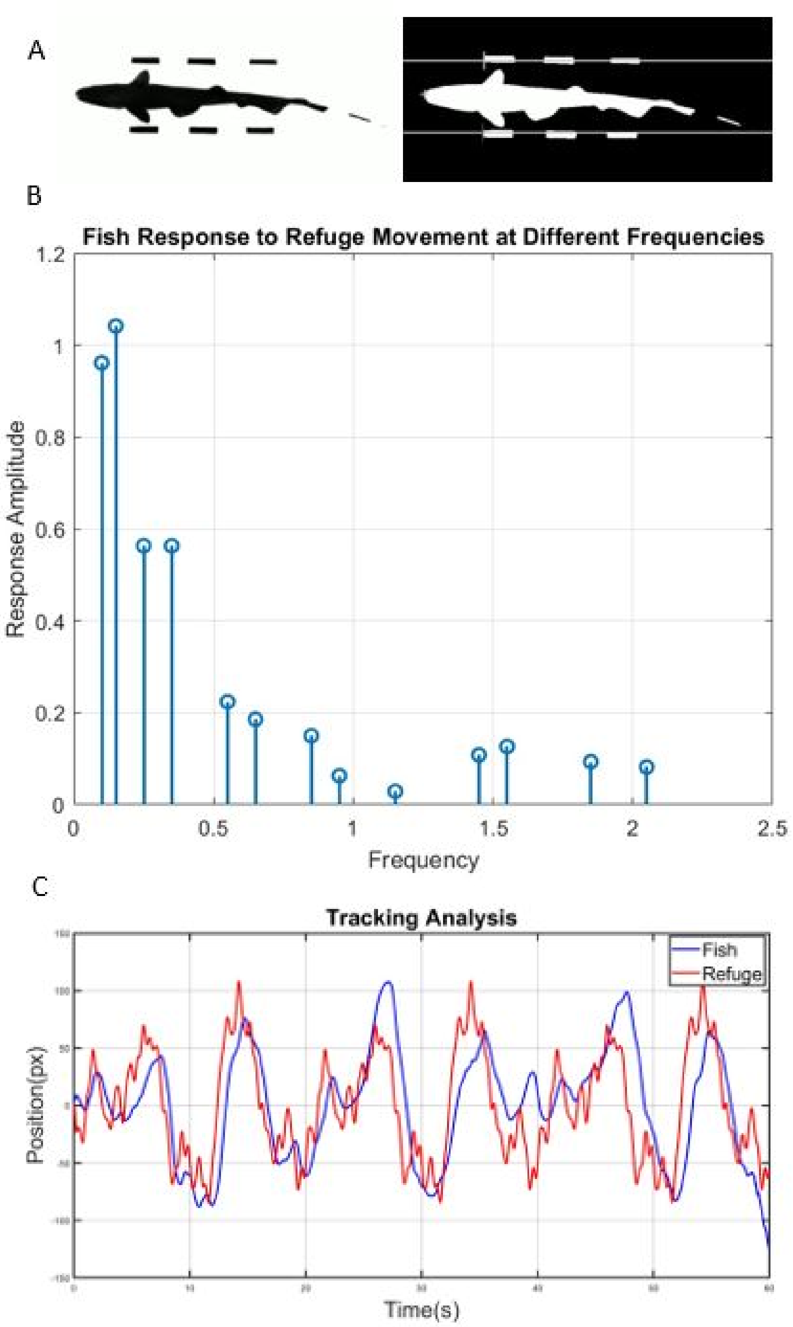


Figure 3.10 In Figure, a video image capturing a black ghost knifefish specimen and the head of the fish marked by image processing and the location of the refuge are shown in A. The graphical representation obtained by Fast Fourier Transform analysis is shown in B. Additionally, C shows the time domain tracking graph showing the fish's behavior regarding refuge tracking.

In Figure 3.10, a video image capturing a black ghost knifefish specimen and the head of the fish marked by image processing and the location of the refuge are shown in A. The graphical representation obtained by Fast Fourier Transform analysis is shown in B. Additionally, C shows the time domain tracking graph showing the fish's behavior regarding refuge tracking [8].

First, we graphically showed the trials that were conducted under all conditions for each fish species in the time domain depicted in Figure 3.11.

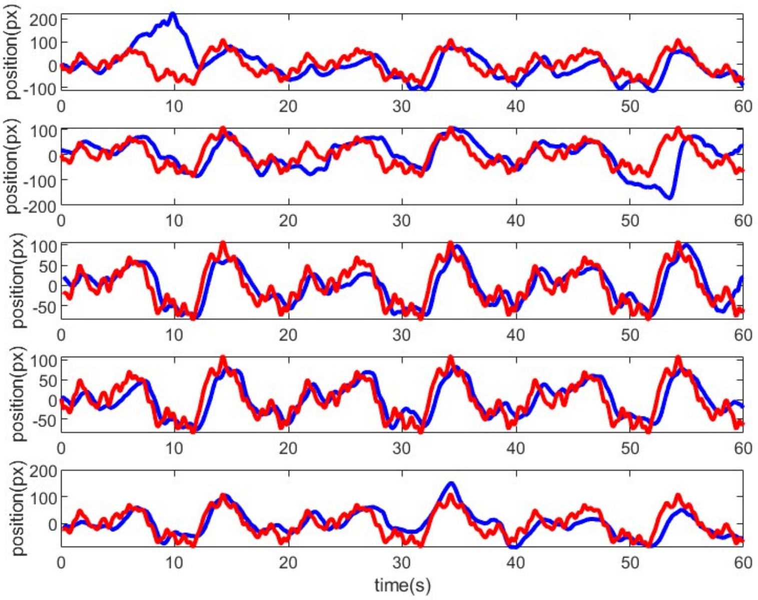


Figure 3.11 5 trial analysis examples performed under one of 54 different conditions for Black ghost knifefish are displayed. While the movement of the refuge shown in red; The movements of the fish are shown in blue.

Next, we plotted the trials under each experimental condition to be analyzed on one graph and their averages on a separate graph depicted in Figure 3.12.

Then, we extracted the frequency response function depicted in Figure 3.13 to analyze the responses of these tracking responses in the time domain in the frequency domain. We plotted the gain and phase values for each condition.

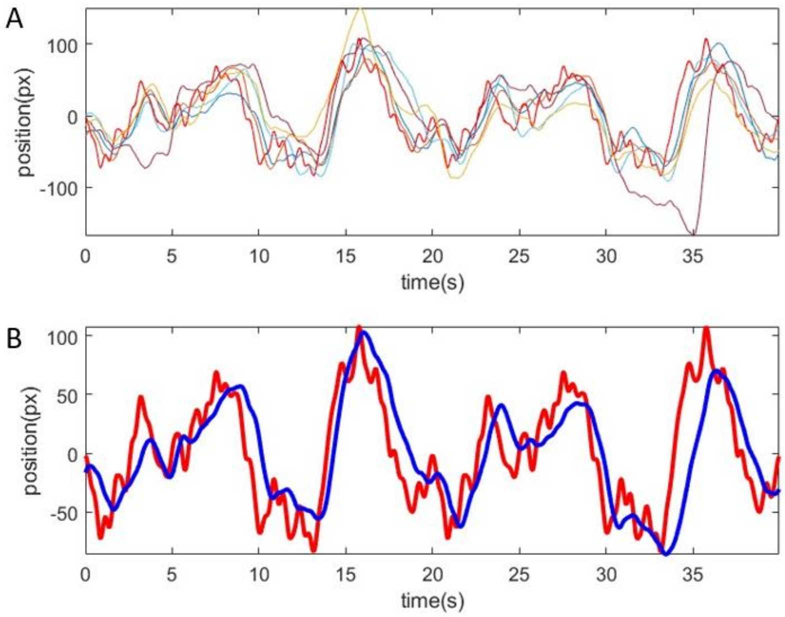


Figure 3.12 Black ghost knifefish refuge tracking in time domain with all trials in one condition. A displays 5 trials in one condition, while B displays average fish and average refuge data.

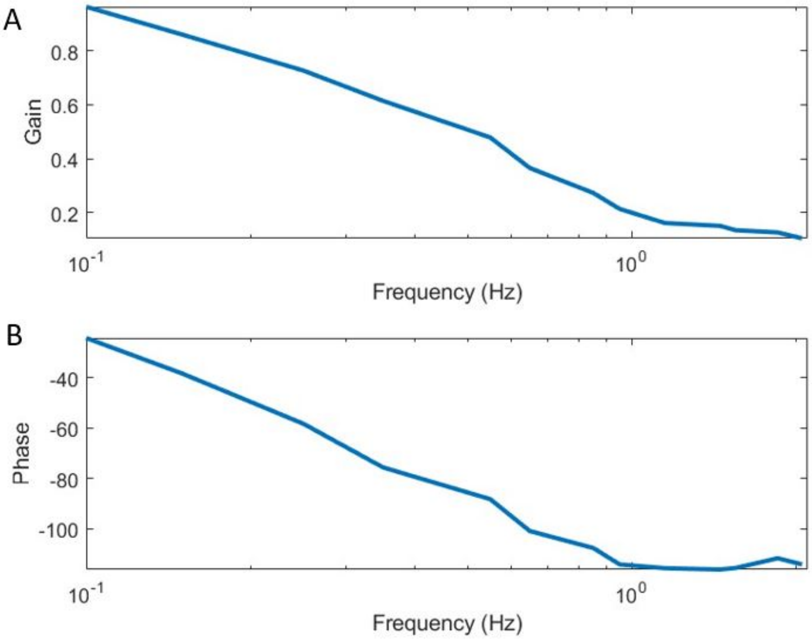


Figure 3.13 From the graphs in the Figure, which serve as the performance measure for the comparative analysis of sensory conditions, the gain in A represents the magnitude of the fish's frequency response; the phase in B represents the time shift.

4. RESULTS

4.1. A Frequency Response Function (FRF) Chart in Black Ghost Knifefish and Glass Knifefish

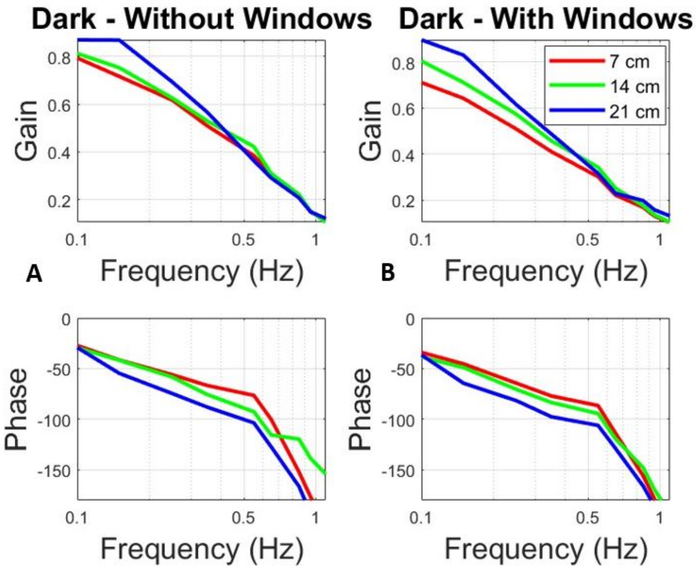


Figure 4.1 The tracking performance of black ghost knifefish of different lengths is shown in the windowless refuge in A and the windowed refuge in B under dark illumination conditions.

In the refuge tracking task, the fish responded at the same frequency in response to the refuge frequency, indicating that the fish could track the stimulus and its movements were not random (see Figure 3.10.B) This pattern of behavior is a result observed for each stimulus category, including sine wave stimuli and sum of sine stimuli previously tested [7].

In accordance with prior research findings, [4, 7, 8, 15] it was observed that fish demonstrated comparable performance in terms of linear tracking. When comparing tracking performance in light conditions with tracking performance in dark conditions, the fish exhibited increased frequency response gains and reduced phase delays.

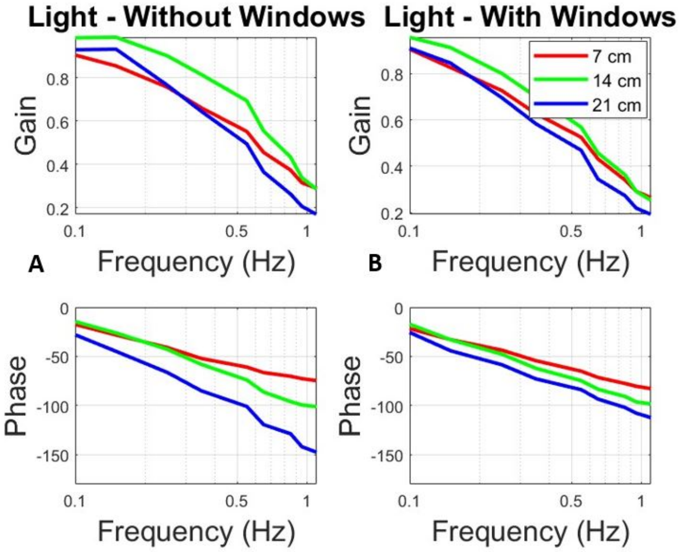


Figure 4.2 The tracking performance of black ghost knifefish of different lengths is shown in the windowless refuge in A and the windowed refuge in B under light illumination conditions.

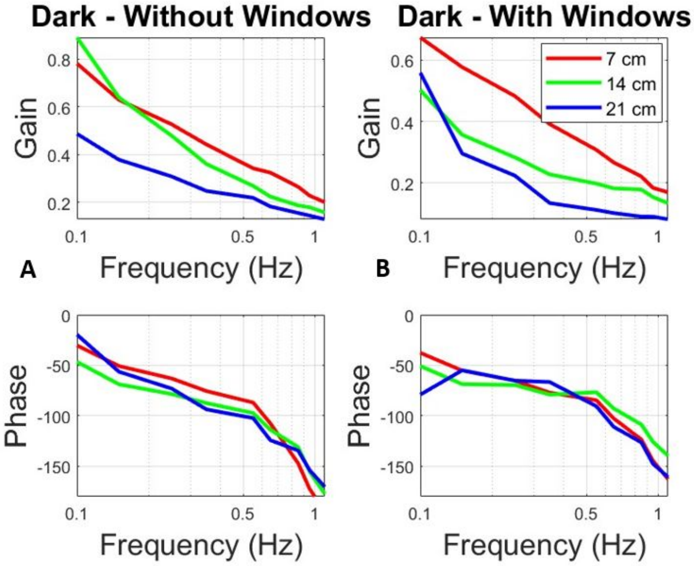


Figure 4.3 The tracking performance of glass knifefish of different lengths is shown in the windowless refuge in A and the windowed refuge in B under dark illumination conditions.

As seen in the Figure 4.3, a large difference in gain emerged as the refuge length increased in windowed and windowless refuges under dark illumination conditions. It is observed that the gain in the windowed refuge is higher than in the windowless refuge.

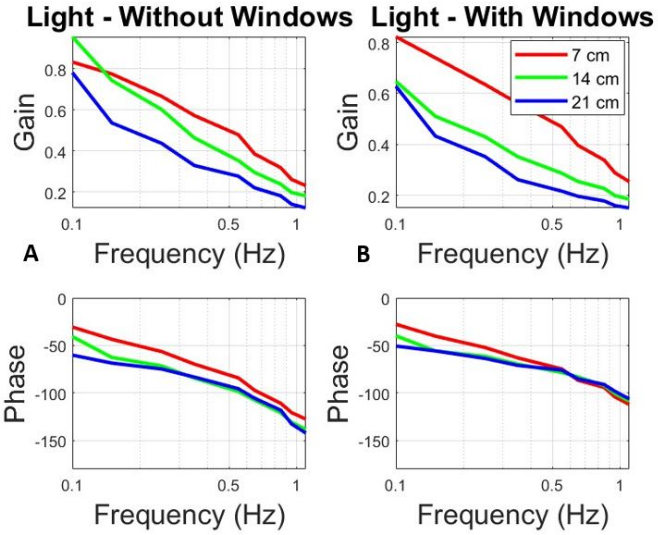


Figure 4.4 The tracking performance of glass knifefish of different lengths is shown in the windowless refuge in A and the windowed refuge in B under light illumination conditions.

We observed a visible deterioration in the fish's ability to track refuge with the transition from the light illumination condition to the dark illumination condition. Specifically, an overall increase in phase delay was observed in dark conditions, particularly at higher frequencies.

While the phase delay generally shows a smoother decrease in the light illumination condition; in the dark illumination condition, a time delay was experienced with a sharp decrease in phase after a certain frequency point.

Notably, a distinct threshold was observed at a frequency point of 0.55, beyond which the gain decreased significantly, and the phase delay exhibited a substantial increase.

The length of refuges significantly affects tracking performance. In all weakly electric fish species, shorter refuges exhibit higher gain, while longer refuges cause more phase delay under similar conditions.

4.2. The Effect of Fish Species and Experimental Conditions on RMSE Values

We performed a mixed-design factorial ANOVA using the RMSE values we calculated based on the average fish trials in each experimental condition. Fish species was our between-subject factor; whereas illumination, conductivity, length, and window were within-subject factors.

4.2.1. Between Subject Effects: Black Ghost Knifefish vs. Glass Knifefish

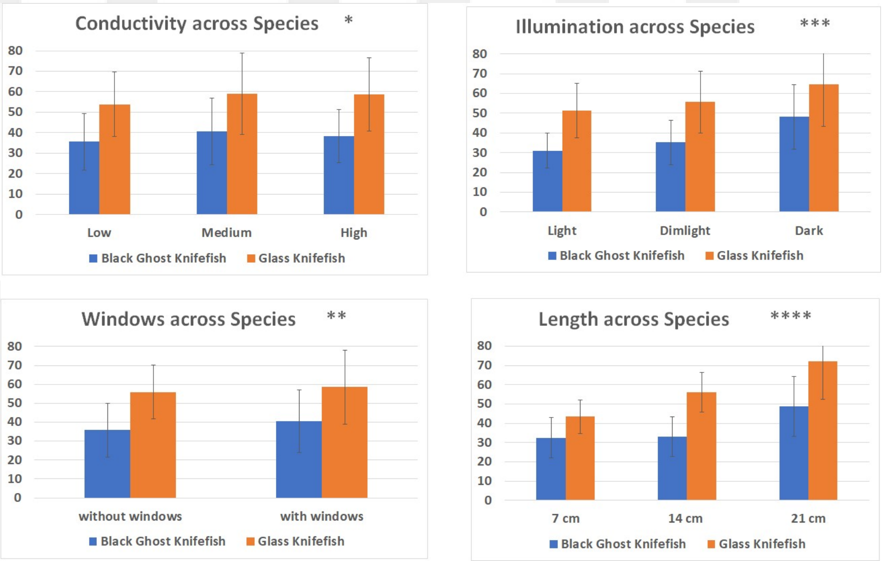
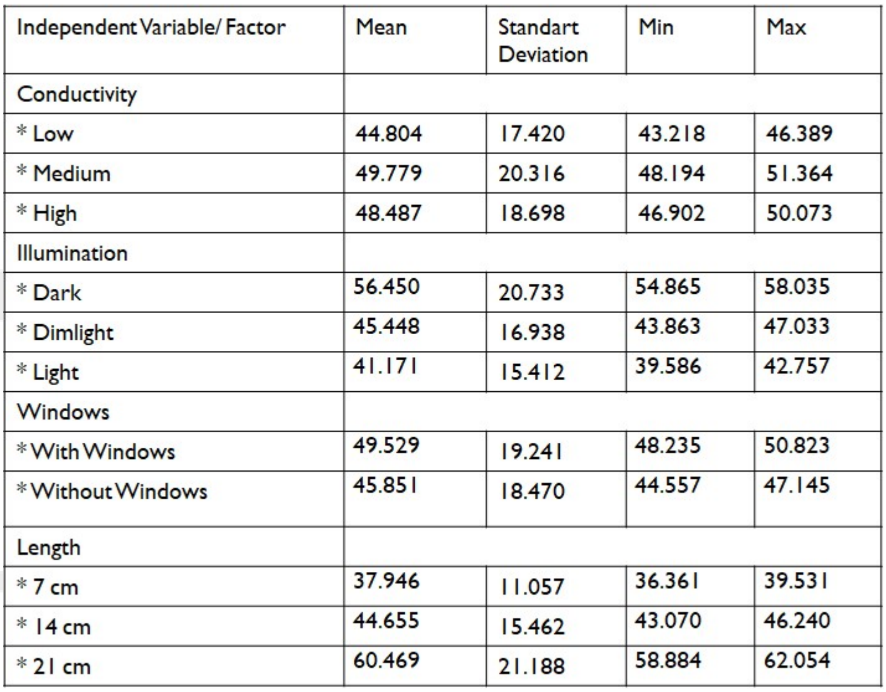


Figure 4.5 Experimental combinations are indicated in the title and refuge lengths are indicated by colors. Error bars show the standard deviation. A mixed design five-way ANOVA, supporting the graph analysis of the species versus 4 different salience conditions, revealed significant effects of refuge length (**** $p < 0.000000005$), illumination (***$p < 0.0005$), the presence or absence of windows (*$p = 0.007$) and conductivity (**$p = 0.023$)

This analysis revealed significant differences in refuge tracking performance between two species (F(1,486) = 48.774 $p = 0.000114$), with black ghost knifefish (M = 38.155 , SE = 1.931) having lower RMSE values than glass knifefish species (M = 57.225 , SE = 1.931). Likewise, it revealed that the most important parameter for tracking performance was the length of the refuge (F(2,486) = 84.151 $p = 3.2265E-9$, followed by illumination (F(2,486) = 44.243 $p = 3.0234E-7$). We observed that conductivity (F(2,486) = 4.838 $p = 0.023$) and windows (F(1,486) = 13.174 $p = 0.007$) had a significant effects on tracking performance (see Figure 4.5).

4.2.2. Within Subject Effect: Conductivity, Illumination, Window and Length



Independent Variable/ Factor	Mean	Standart Deviation	Min	Max
Conductivity				
* Low	44.804	17.420	43.218	46.389
* Medium	49.779	20.316	48.194	51.364
* High	48.487	18.698	46.902	50.073
Illumination				
* Dark	56.450	20.733	54.865	58.035
* Dimlight	45.448	16.938	43.863	47.033
* Light	41.171	15.412	39.586	42.757
Windows				
* With Windows	49.529	19.241	48.235	50.823
* Without Windows	45.851	18.470	44.557	47.145
Length				
* 7 cm	37.946	11.057	36.361	39.531
* 14 cm	44.655	15.462	43.070	46.240
* 21 cm	60.469	21.188	58.884	62.054

Figure 4.6 Estimated Marginal Means

There was a statistically significant difference across all illumination conditions when compared pairwise using Bonferroni correction, $p = 0.001$ that is between Dark and Dimlight; $p = 0.000$ between Dark and Light; $p = 0.048$ between Dimlight and Light.

There was a statistically significant difference between all length types $p = 0.007$ between 7 cm and 14 cm; $p = .000019$ between 7 cm and 21 cm; $p = 0.000026$ between 14 cm and 21 cm. Whether the refuge had a window or not made a statistically significant difference ($p = 0.007$). Pairwise comparisons with Bonferroni correction showed that there is a statistically significant difference only between low and medium conductivity conditions ($p = 0.05$). (see Figure 4.6).

For significant interaction effects, we performed follow-up using Bonferroni corrections. There was a significant interaction between Species and Length (F(2,486) = 8.002 $p = 0.004$) Illumination and Windows (F(2,486) = 4.658 $p = 0.025$) and Illumination and Length (F(2,486) = 6.671 $p = 0.001$).

Length	**Black Ghost Knifefish**	**Glass Knifefish**
7 cm	M = 32.527, SE = 2.316	M = 43.365, SE = 2.316
14 cm	M = 33.159, SE = 2.010	M = 56.151, SE = 2.010
21 cm	M = 48.778, SE = 2.852	M = 72.160, SE = 2.852

Table 4.1 Species and Length interactions over RMSE Values

There was a statistically significant difference between refuge lengths of 7 cm and 21 cm $p = 0.002$ and refuge lengths of 14 cm and 21 cm $p = 0.000$ in black ghost knifefish species. We did not observe a significance between between 7 cm and 14 cm refuge lengths.

However, there was a statistically significant difference between all refuge lengths in glass knifefish species. Statistical significance between 7 cm and 14 cm $p = 0.001$; between 14 cm and 21 cm and between 7 cm and 21 cm, statistical significance was $p = 0.000$ (see Table 4.1 and Figure 4.7-A).

The analysis showed that testing in windowed and windowless conditions made a significant difference only for black ghost knifefish species, $p = 0.010$. Performances under window conditions were similar for glass knifefish species (see Figure 4.7-B).

Length	**Illumination**		
	Dark	Dimlight	Light
7 cm	M = 43.607, SE = 2.107	M = 36.393, SE = 1.872	M = 33.837, SE = 1.467
14 cm	M = 52.959, SE = 1.700	M = 42.635, SE = 1.614	M = 38.371, SE = 1.408
21 cm	M = 72.784, SE = 3.978	M = 57.316, SE = 2.227	M = 51.306, SE = 1.372

Table 4.2 Illumination and Length Interactions over RMSE Values

Statistical difference between 7 cm and 21 cm refuge lengths in ‘Dark’ illumination condition $p = 0.000$; the statistical difference between 14 cm and 21 cm $p = 0.001$ and between 7 cm and 14 cm is $p = 0.002$.

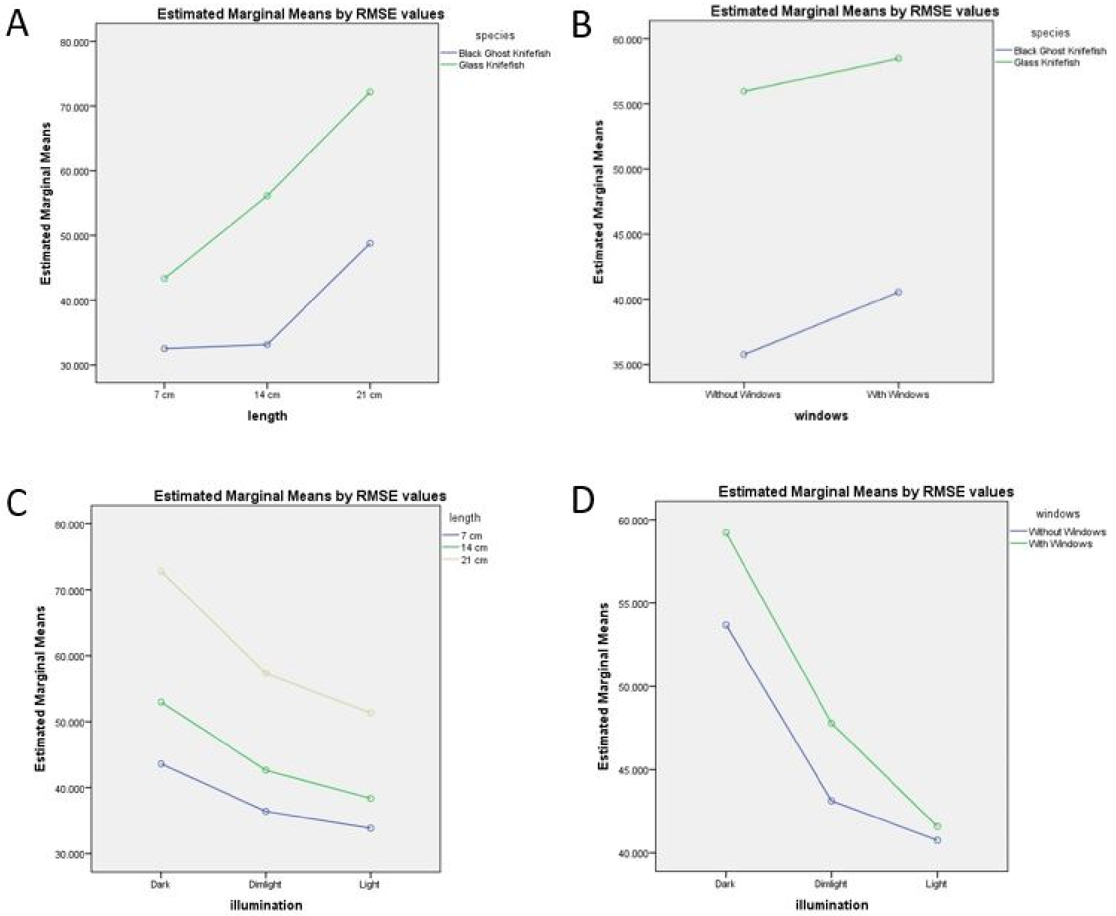


Figure 4.7 The graphs show the interactions of ‘Species and Length’ in A, ‘Species and Windows’ in B, ‘Illumination and Length’ in C and ‘Illumination and Windows’ in D using Bonferroni corrections

While the statistical difference between 7 cm and 14 cm refuge lengths in ‘Dimlight’ illumination conditions was $p = 0.033$; the statistical difference between 7 cm and 21 cm and between 14 cm and 21 cm was $p = 0.000$. Finally, the statistical difference between 7 cm and 21 cm $p = 0.000078$ and between 14 cm and 21 cm refuge lengths $p = 0.000008$ in the ‘Light’ illumination condition, while we did not observe a significance between between 7 cm and 14 cm refuge lengths (see Table 4.2 and Figure 4.7-C).

If the illumination condition was ‘Dark’, the difference with and without windows was statistically significant $p = 0.015$; if the illumination condition was ‘Dimlight’, the difference with and without windows was statistically significant $p = 0.003$.

Windows	**Illumination**		
	Dark	Dimlight	Light
WithoutWindows	M = 53.681	M = 43.115	M = 40.758
	SE = 2.409	SE = 1.556	SE = 1.109
WithWindows	M = 59.219	M = 47.782	M = 41.585
	SE = 2.415	SE = 1.685	SE = 1.252

Table 4.3 Illumination and Windows Interactions over RMSE Values

It was observed that the presence or absence of windows did not make a statistical difference in the ‘Light’ illumination condition (see Table 4.3 and Figure 4.7-D).

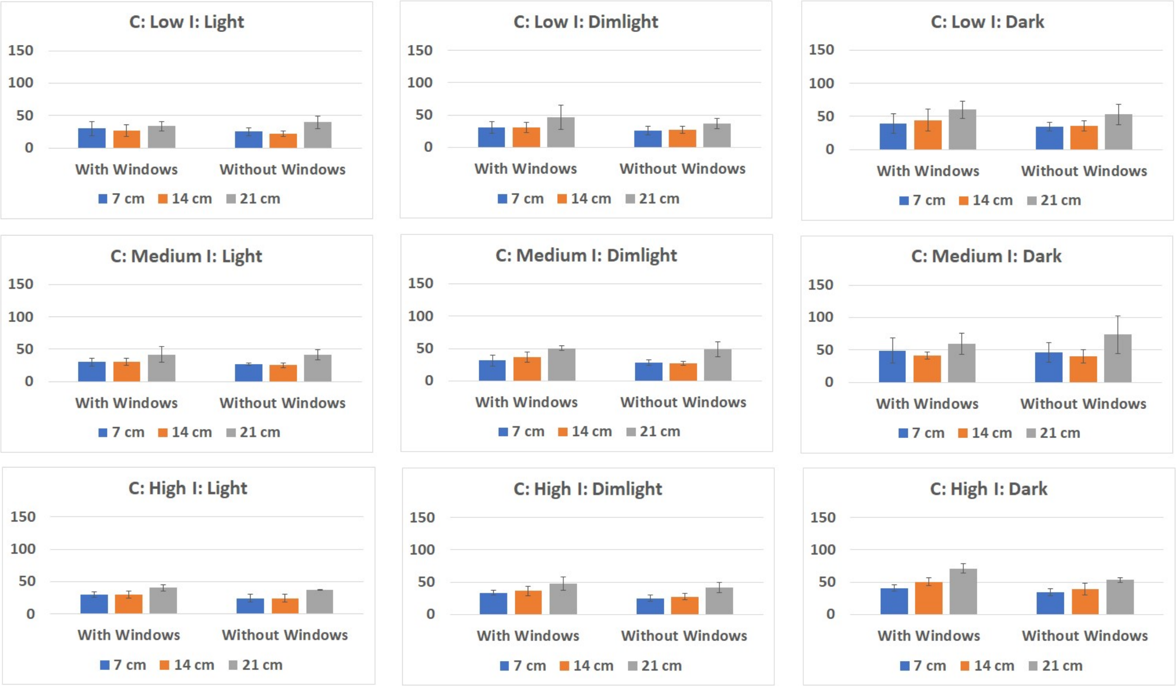


Figure 4.8 RMSE Values in Black Ghost Knifefish for 54 Different Conditions

As an overall evaluation, both black ghost knifefish and glass knifefish showed tracking performance in parallel with refuge lengths. A gradually increasing RMSE and naturally deteriorating tracking performance was demonstrated as we moved from 7 cm refuge lengths to 21 cm refuge lengths.

Figure 4.9 RMSE Values in Glass Knifefish for 54 Different Conditions

In addition, black ghost knifefish performed better in each of the 54 different sensory salience conditions compared to glass knifefish (see Figure 4.8 and 4.9).

4.3. The Effect of Fish Species and Experimental Conditions on Tracking Error with the Velocity Values

A gradient represents the rate of change of a quantity over a certain range. When applied to position data over time, it can be used to estimate velocity. We used the gradient function in Matlab to calculate the gradient of our fish position data over time values.

4.3.1. Between Subject Effects: Black Ghost Knifefish vs. Glass Knifefish

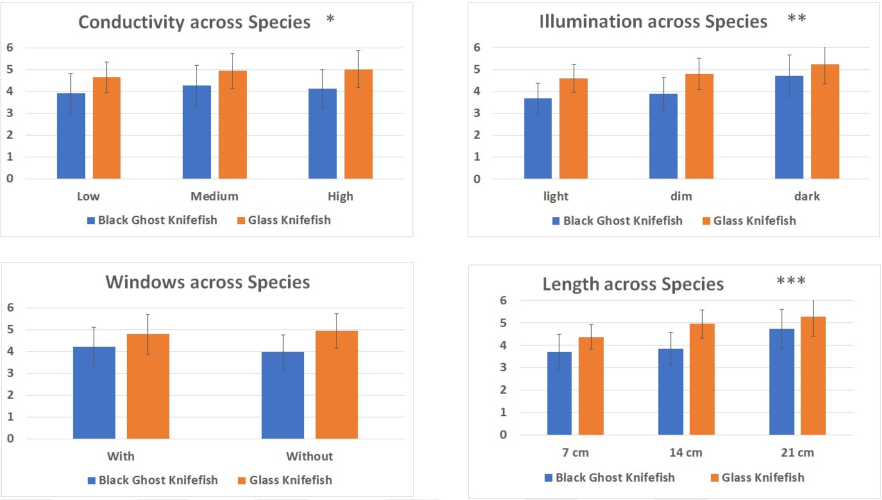
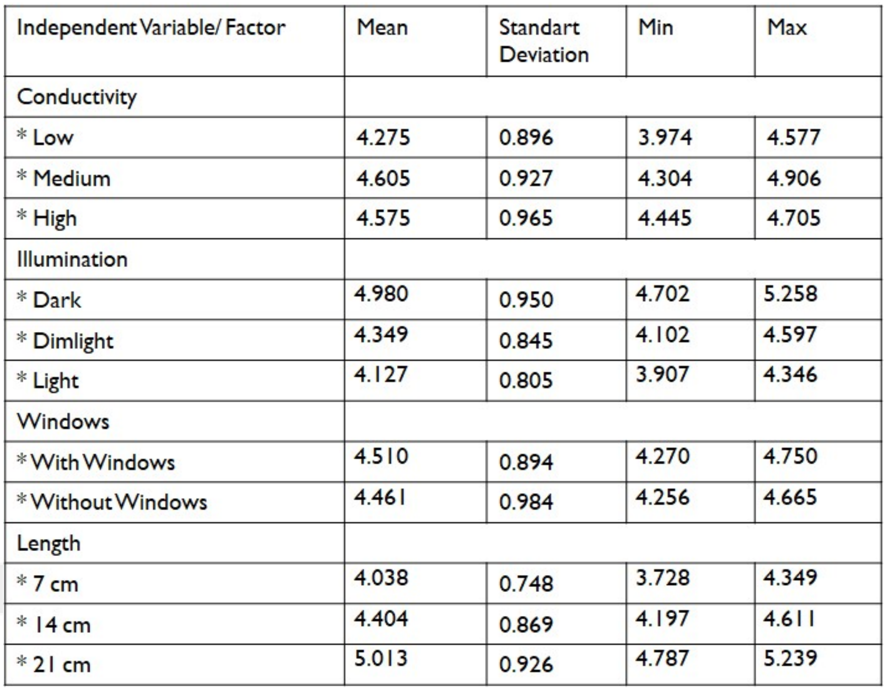


Figure 4.10 Experimental combinations are indicated in the title and refuge lengths are indicated by colors. Error bars show the standard deviation.A mixed design five-way ANOVA, supporting the graph analysis of the species versus 4 different salience conditions, revealed significant effects of refuge length (*** $p < 0.00000005$), illumination (**$p < 0.0000005$) and conductivity (*$p < 0.05$)

This analysis revealed significant differences in refuge tracking performance between two species (F(1,486) = 16.683 $p = 0.004$), with black ghost knifefish (M = 4.099 , SE = .134) having lower tracking error than glass knifefish species (M = 4.871 , SE = .134). Likewise, it revealed that the most important parameter for tracking performance was the length of the refuge (F(2,486) = 53.733 $p = 7.9534E{-}8$, followed by illumination (F(2,486) = 46.932 $p = 2.0236E - 7$. We assessed that conductivity had a significant effect on tracking performance (F(2,486) = 6.328 $p = 0.009$) and it was observed that the window condition did not reveal a statistically significant difference (see Figure 4.10).

4.3.2. Within Subject Effect: Conductivity, Illumination, Window and Length

When we evaluate the illumination factor, the difference between dark and dimlight ($p = 0.000069$) and between dark and light was statistically significant ($p = 0.000207$), while the difference between dimlight and light was not significant. We found that there was a statistically significant difference between refuge length conditions for all length types ($p =$



Independent Variable/ Factor	Mean	Standart Deviation	Min	Max
Conductivity				
* Low	4.275	0.896	3.974	4.577
* Medium	4.605	0.927	4.304	4.906
* High	4.575	0.965	4.445	4.705
Illumination				
* Dark	4.980	0.950	4.702	5.258
* Dimlight	4.349	0.845	4.102	4.597
* Light	4.127	0.805	3.907	4.346
Windows				
* With Windows	4.510	0.894	4.270	4.750
* Without Windows	4.461	0.984	4.256	4.665
Length				
* 7 cm	4.038	0.748	3.728	4.349
* 14 cm	4.404	0.869	4.197	4.611
* 21 cm	5.013	0.926	4.787	5.239

Figure 4.11 Estimated Marginal Means for Tracking Error(Velocity)

0.020 between 7 cm and 14 cm; $p = 0.000024$ between 7 cm and 21 cm and $p = 0.000332$ between 14 cm and 21 cm). There was a statistically significant difference only between low and medium conductivity ($p = 0.016$). Whether the refuge had a window or not did not make a statistically significant difference (see Figure 4.11).

We performed follow-up tests for binary interactions using Bonferroni corrections. We can say that there is a significant interaction between Species and Windows (F(1,486) = 23.049 $p = 0.001$), Species and Length (F(2,486) = 4.431 $p = 0.029$) and Illumination and Windows (F(2,486) = 8.226 $p = 0.003$).

Length	**Black Ghost Knifefish**	**Glass Knifefish**
7 cm	M = 3.705, SE = .190	M = 4.372, SE = .190
14 cm	M = 3.858, SE = .127	M = 4.950, SE = .127
21 cm	M = 4.734, SE = .139	M = 5.291, SE = .139

Table 4.4 Species and Length Interactions over Tracking Error(Velocity)

There was a statistically significant difference between refuge lengths of 7 cm and 21 cm $p = 0.000205$ and refuge lengths of 14 cm and 21 cm $p = 0.000293$ in black ghost knifefish

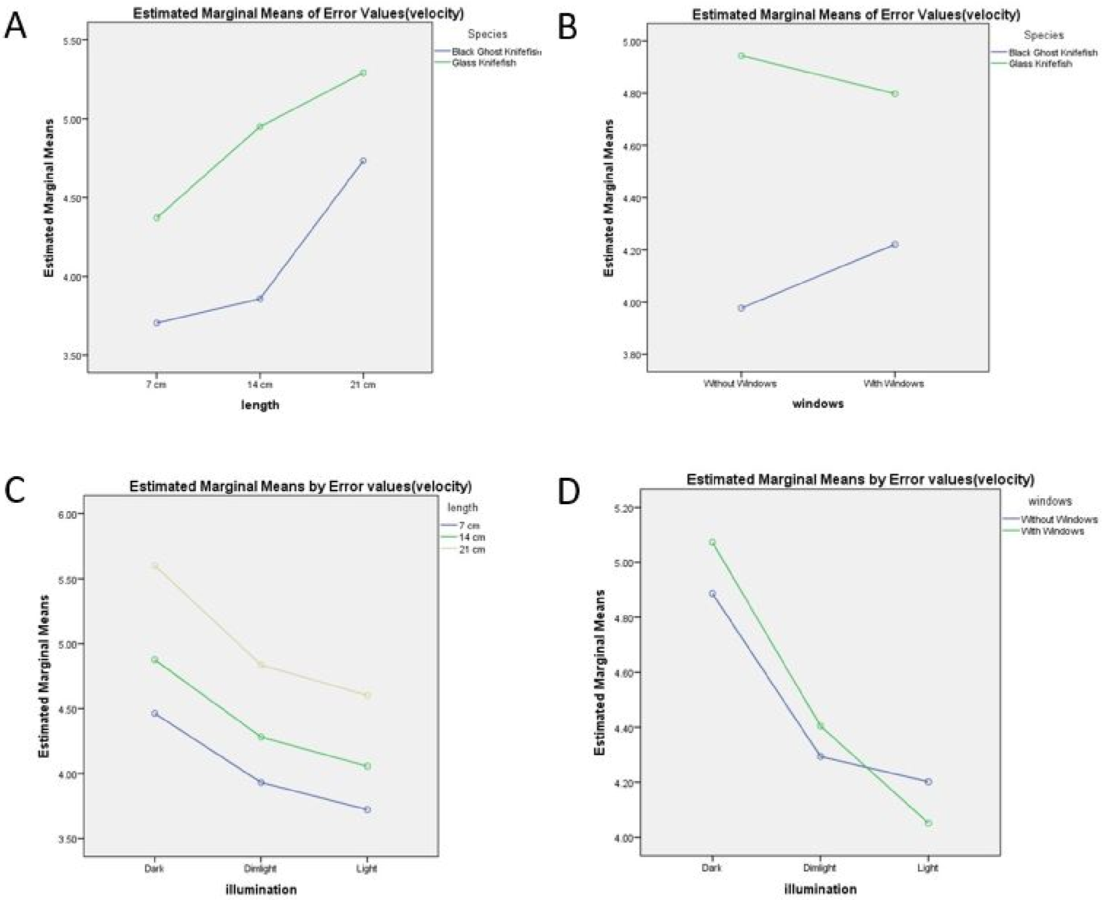


Figure 4.12 The graphs show the interactions of 'Species and Length' in A, 'Species and Windows' in B, 'Illumination and Length' in C and 'Illumination and Windows' in D using Bonferroni corrections

species. We did not observe a significance between between 7 cm and 14 cm refuge lengths. There was a statistically significant difference between refuge lengths of 7 cm and 14 cm $p = 0.011$ and refuge lengths of 7 cm and 21 cm $p = 0.000$ in glass knifefish species. We did not observe a significance between between 14 cm and 21 cm refuge lengths (see Table 4.4 and Figure 4.12-A).

Statistical difference between 7 cm and 21 cm refuge lengths in 'Dark' illumination condition $p = 0.000$; the statistical difference between 14 cm and 21 cm $p = 0.004$ and between 7 cm and 14 cm was $p = 0.035$. While the statistical difference between 7 cm and 21 cm refuge lengths in 'Dimlight' illumination conditions was $p = 0.000$; the statistical difference

Length	**Illumination**		
	Dark	Dimlight	Light
7 cm	M = 4.463, SE = .133	M = 3.931, SE = .152	M = 3.722, SE = .135
14 cm	M = 4.876, SE = .106	M = 4.281, SE = .097	M = 4.056, SE = .118
21 cm	M = 5.601, SE = .188	M = 4.836, SE = .114	M = 4.602, SE = .085

Table 4.5 Illumination and Length Interactions over Tracking Error(Velocity)

between 7 cm and 14 cm $p = 0.035$ and between 14 cm and 21 cm was $p = 0.001$. Finally, the statistical difference between 7 cm and 21 cm $p = 0.000455$ and between 14 cm and 21 cm refuge lengths $p = 0.000326$ in the 'Light' illumination condition, while there was no statistically significant difference between 7 cm and 14 cm refuge lengths (see Table 4.5 and Figure 4.12-C).

Windows	**Illumination**		
	Dark	Dimlight	Light
Without Windows	M = 4.886, SE = .121	M = 4.294, SE = .111	M = 4.202, SE = .085
With Windows	M = 5.073, SE = .131	M = 4.405, SE = .110	M = 4.051, SE = .114

Table 4.6 Illumination and Windows Interactions over Tracking Error(Velocity)

If the illumination condition was 'Dark' and 'Light', we observed that the difference with and without windows was statistically significant ($p < 0.05$); it was observed that the presence or absence of windows did not make a statistical difference in the 'Dimlight' illumination condition (see Table 4.6 and Figure 4.12-D).

When we evaluated the results graphically according to the tracking error in 54 sensory salience conditions, it was seen that the tracking performance of black ghost knifefish was better than glass knifefish (see Figure 4.13 and Figure 4.14).

Figure 4.13 Tracking Error(Velocity) in Black Ghost Knifefish for 54 Different Conditions

Figure 4.14 Tracking Error(Velocity) in Glass Knifefish for 54 Different Conditions

4.4. The Effect of Fish Species and Experimental Conditions on Tracking Error with the Position Values

We performed a mixed-design factorial ANOVA using the position values based on the tracking error that we calculated based on the average fish trials in each experimental condition. Tracking error, denoted as ϵ, is quantified as the spatial separation in the complex plane between the actual frequency response *H(s)* of the system and the desired ideal tracking conditions characterized by a gain of 1 and a phase of 0 degrees.

$$\epsilon(s) = ||H(s) - (1 + 0i)||$$

Fish species was our between-subject factor; whereas illumination, conductivity, length, and window were within-subject factors.

4.4.1. Between Subject Effects: Black Ghost Knifefish vs. Glass Knifefish

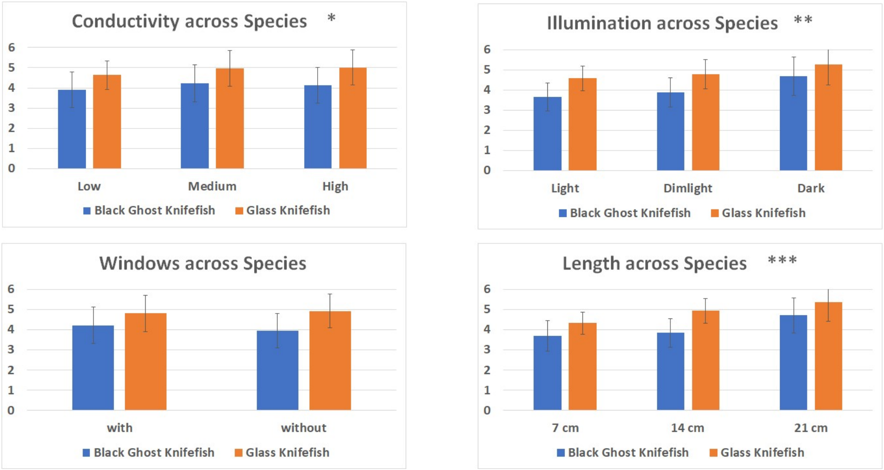
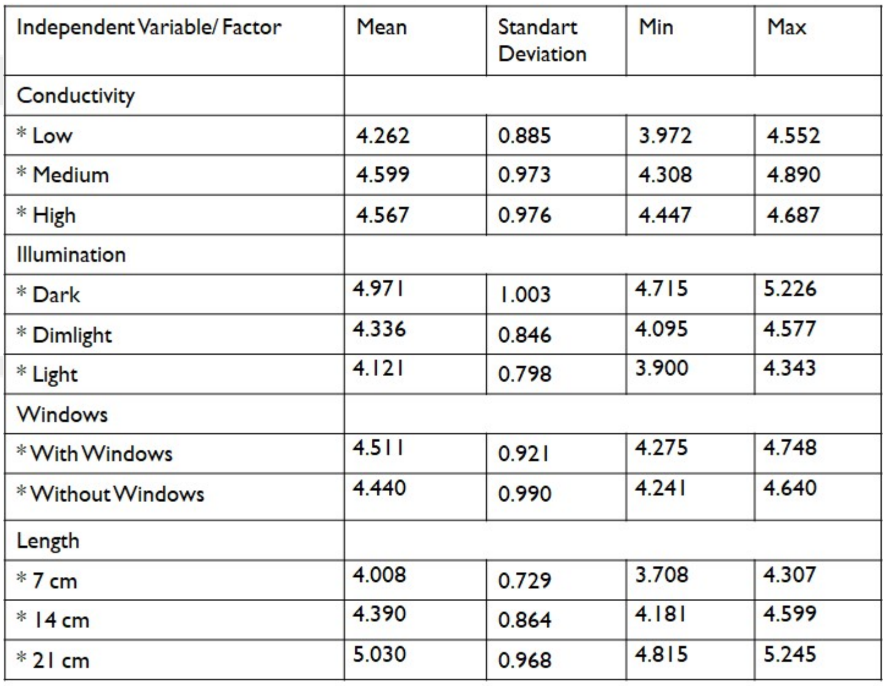


Figure 4.15 Experimental combinations are indicated in the title and refuge lengths are indicated by colors. Error bars show the standard deviation. A mixed design five-way ANOVA, supporting the graph analysis of the species versus 4 different salience conditions, revealed significant effects of refuge length (*** $p < 0.000000005$), illumination (**$p < 0.00000005$) and conductivity (*$p < 0.05$)

This analysis revealed significant differences in refuge tracking performance between two species (F(1,486) = 18.398 $p = 0.003$), with black ghost knifefish (M = 4.083 , SE = .130) having lower tracking error than glass knifefish species (M = 4.869 , SE = .130).

Likewise, it revealed that the most important parameter for tracking performance was the length of the refuge (F(2,486) = 62.339 $p = 2.7999E{-}8$), followed by illumination (F(2,486) = 54.444 $p = 7.2574E{-}8$). We assessed that conductivity had a significant effect on tracking performance (F(2,486) = 7.498 $p = 0.005$) and it was observed that the window situation did not reveal a statistically significant difference (see Figure 4.15).



Independent Variable/ Factor	Mean	Standart Deviation	Min	Max
Conductivity				
* Low	4.262	0.885	3.972	4.552
* Medium	4.599	0.973	4.308	4.890
* High	4.567	0.976	4.447	4.687
Illumination				
* Dark	4.971	1.003	4.715	5.226
* Dimlight	4.336	0.846	4.095	4.577
* Light	4.121	0.798	3.900	4.343
Windows				
* With Windows	4.511	0.921	4.275	4.748
* Without Windows	4.440	0.990	4.241	4.640
Length				
* 7 cm	4.008	0.729	3.708	4.307
* 14 cm	4.390	0.864	4.181	4.599
* 21 cm	5.030	0.968	4.815	5.245

Figure 4.16 Estimated Marginal Means for Tracking Error(Position)

In the illumination factor, the difference between dark and dimlight ($p = 0.000032$) and between dark and light was statistically significant ($p = 0.000124$), while we did not observe a significance difference between dimlight and light illumination conditions. We found that there was a statistically significant difference between refuge length conditions for all length types ($p = 0.010$ between 7 cm and 14 cm; $p = 0.000018$ between 7 cm and 21 cm and $p = 0.000218$ between 14 cm and 21 cm). There was a statistically significant difference between low and medium conductivity ($p = 0.013$) and between low and high conductivity

($p = 0.038$). However, there was no statistically significant difference between medium and high conductivity levels. Whether the refuge had a window or not did not make a statistically significant difference (see Figure 4.16).

4.4.2. Within Subject Effect: Conductivity, Illumination, Window and Length

We performed follow-up tests for binary interactions using Bonferroni corrections. We found that between Windows and Species(F(2,486) = 15.872 $p = 0.004$) Length and Species (F(2,486) = 4.024 $p = 0.038$) Illumination and Windows (F(2,486) = 7.095 $p = 0.006$) Illumination and Length (F(2,486) = 3.853 $p = 0.011$) statistically significant difference was considered.

Length	Black Ghost Knifefish	Glass Knifefish
7 cm	M = 3.688, SE = .184	M = 4.328, SE = .184
14 cm	M = 3.845, SE = .128	M = 4.935, SE = .128
21 cm	M = 4.715, SE = .132	M = 5.346, SE = .132

Table 4.7 Species and Length Interactions over Tracking Error(Position)

There was a statistically significant difference between refuge lengths of 7 cm and 21 cm ($p = 0.000226$) and refuge lengths of 14 cm and 21 cm ($p = 0.000289$) in black ghost knifefish species. We did not observe a significance between 7 cm and 14 cm refuge lengths. While there was a statistically significant difference ($p = 0.005$) between 7 cm and 14 cm refuge lengths and ($p = 0.029$) between 14 cm and 21 cm refuge lengths in glass knifefish species; there was a statistically significant difference between 7 cm and 21 cm refuge lengths ($p = 0.000$) (see Table 4.7 and Figure 4.17-A).

Windows	Black Ghost Knifefish	Glass Knifefish
Without Windows	M = 3.951, SE = .122	M = 4.930, SE = .122
With Windows	M = 4.214, SE = .145	M = 4.809, SE = .145

Table 4.8 Species and Windows Interactions over Tracking Error(Position)

The difference between windowed refuges and windowless refuges was statistically significant ($p = 0.005$) in only black ghost knifefish (see Table 4.8 and Figure 4.17-B).

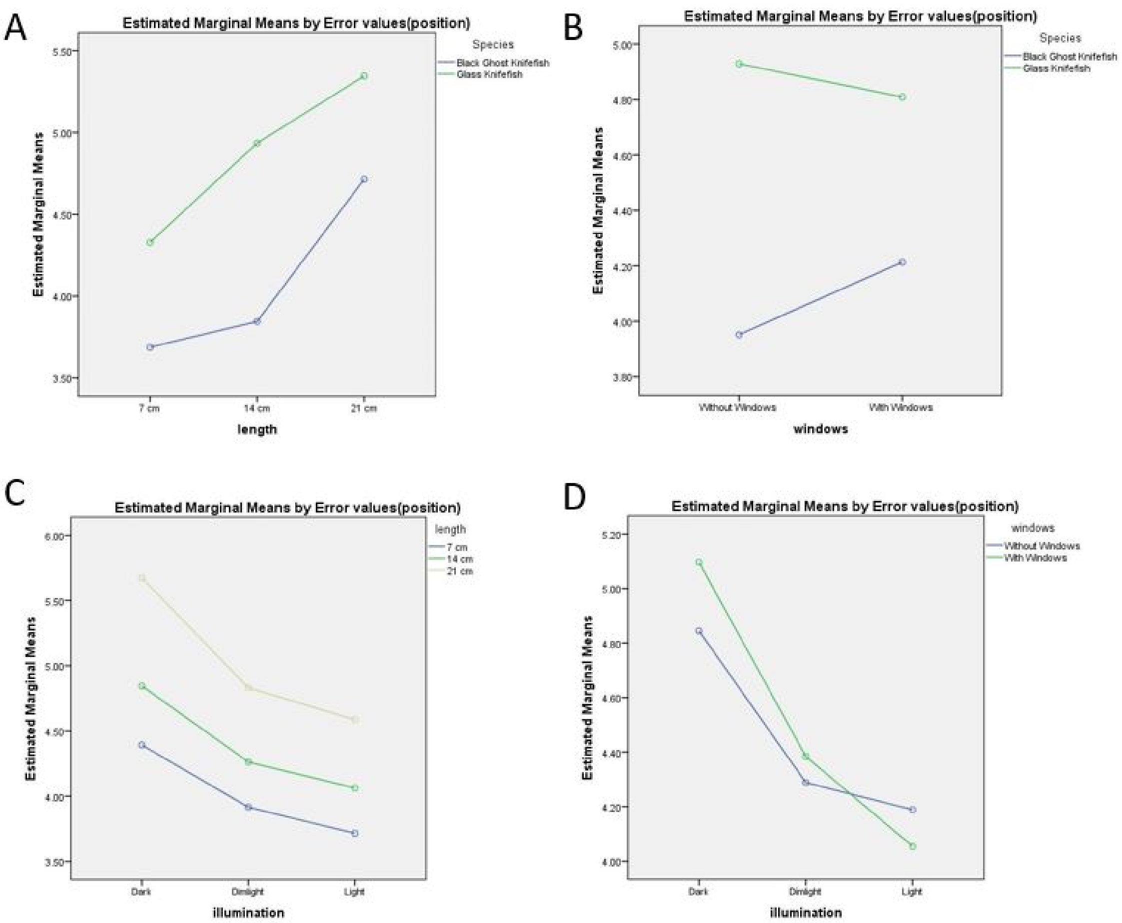


Figure 4.17 The graphs show the interactions of ‘Species and Length’ in A, ‘Species and Windows’ in B, ‘Illumination and Length’ in C and ‘Illumination and Windows’ in D using Bonferroni corrections

The statistical difference between 7 cm and 21 cm refuge lengths in ‘Dark’ illumination condition was $p = 0.000$; the statistical difference $p = 0.001$ between 14 cm and 21 cm and between 7 cm and 14 cm $p = 0.009$.

Length	**Illumination**		
	Dark	Dimlight	Light
7 cm	M = 4.392, SE = .120	M = 3.916, SE = .149	M = 3.715, SE = .133
14 cm	M = 4.845, SE = .110	M = 4.262, SE = .096	M = 4.062, SE = .117
21 cm	M = 5.674, SE = .168	M = 4.831, SE = .112	M = 4.587, SE = .084

Table 4.9 Illumination and Length Interactions over Tracking Error(Position)

While the statistical difference between 7 cm and 21 cm refuge lengths in ‘Dimlight’ illumination conditions was $p = 0.000$; the statistical difference $p = 0.035$ between 7 cm and 14 cm and $p = 0.002$ between 14 cm and 21 cm. Finally, the statistical difference $p = 0.000253$ between 7 cm and 21 cm and $p = 0.000249$ between 14 cm and 21 cm refuge lengths in the ‘Light’ illumination condition. The statistical difference between 7 cm and 14 cm was $p = 0.048$ (see Table 4.9 and Figure 4.17-C).

Windows	**Illumination**		
	Dark	Dimlight	Light
Without Windows	M = 4.845, SE = .118	M = 4.288, SE = .108	M = 4.189, SE = .088
With Windows	M = 5.096, SE = .123	M = 4.384, SE = .112	M = 4.054, SE = .113

Table 4.10 Illumination and Windows Interactions over Tracking Error(Position)

If the illumination condition was ‘Dark’, we observed that the difference with and without windows was statistically significant ($p = 0.029$); it was observed that the presence or absence of windows did not make a statistical difference in the ‘Dimlight’ and ‘Light’ illumination condition (see Table 4.10 and Figure 4.17-D).

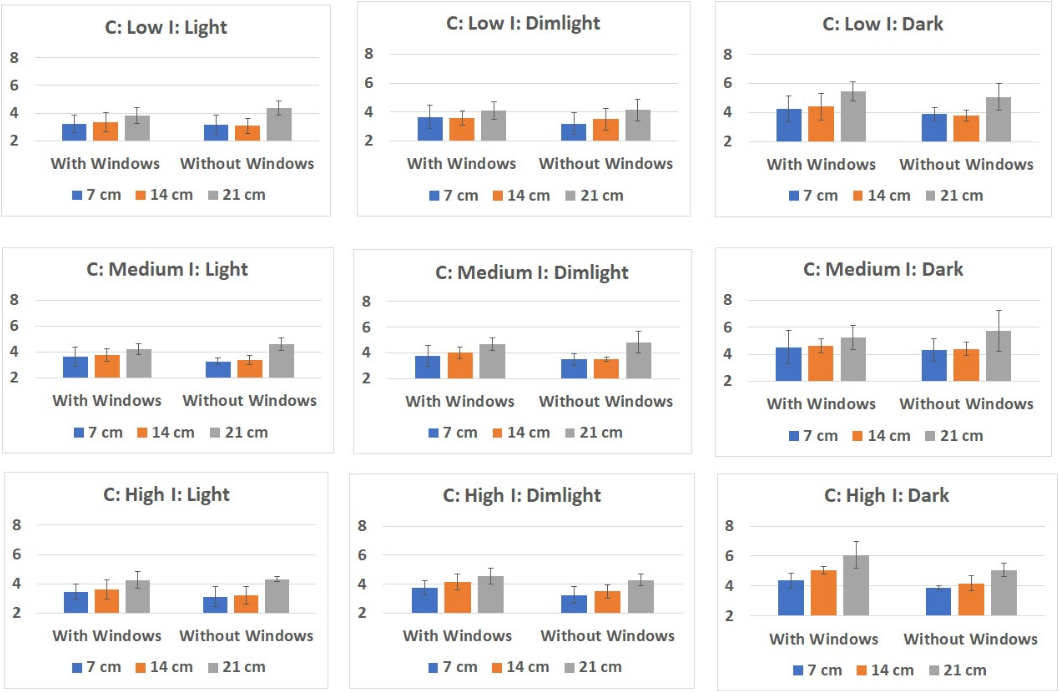


Figure 4.18 Tracking Error(Position) in Black Ghost Knifefish for 54 Different Conditions

When we evaluated the results graphically according to the tracking error in 54 sensory salience conditions, it was seen that the tracking performance of black ghost knifefish was better than glass knifefish (**see Figure 4.18 and Figure 4.19**).

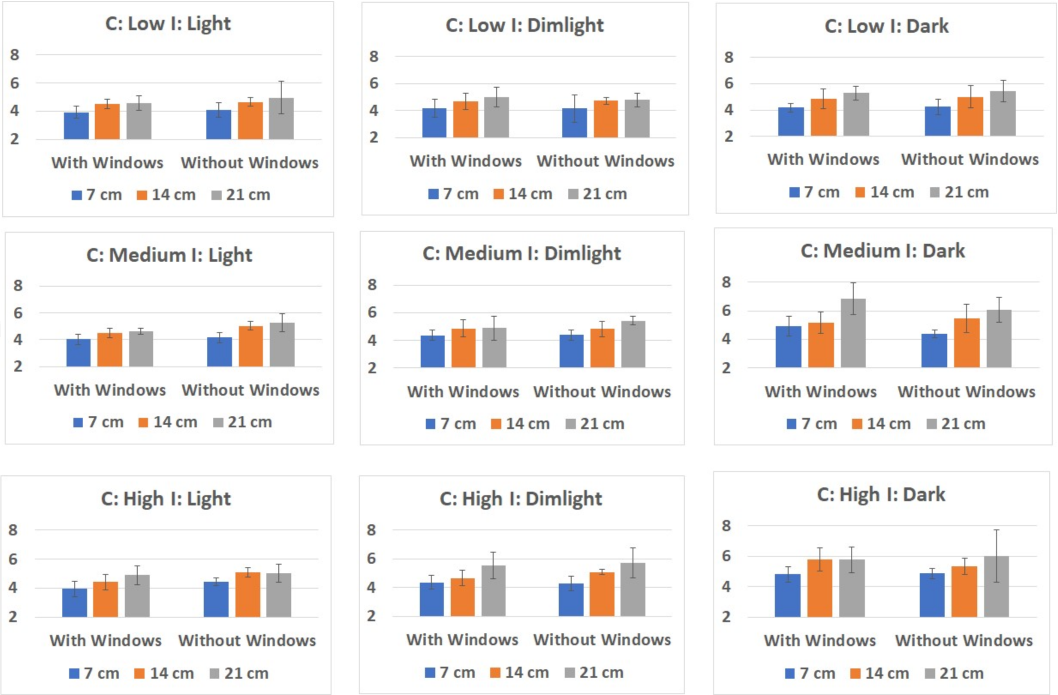


Figure 4.19 Tracking Error(Position) in Glass Knifefish for 54 Different Conditions

4.5. Discussion

In this study, our aim was to examine the performance statistics of refuge tracking behavior under different sensory salience conditions using two different species of weakly electric fishes. To this end, we used two different metrics to measure the fish's refuge tracking performance. One of them was RMSE values and the other was the tracking error.

In our experimental conditions we manipulated illumination, conductivity, window and length. Our specific hypotheses were as follows: 1) we expected that illumination would have a major effect on fish behavior as indicated by RMSE and tracking error. 2) We expected the presence of windows in the refuge to influence the spatial perception and tracking behavior of fish by electrosense. 3) Considering the difference between the length of the fish itself and the length of the refuge, we expected the refuge length to have a large effect on tracking and position perception. 4) Since the conductivity ranges of the water in which the fish are found change from low to high, we thought that it would make a difference in terms of electrical perception and this difference would have a great impact on the results. 5) Finally, we wanted to reveal through our analysis what similarities and differences there would be in refuge-tracking behavior between black ghost knifefish and glass knifefish species, even though they have similar anatomical features.

As an overall assessment, there was a linear relationship between the movements of the fish and the movements of the refuge it followed. Although the refuge tracking movements of the fish were at the same frequency, there were changes in gain and phase [4, 7, 15].

The analysis results revealed that the most important parameters for refuge tracking performance were illumination and length. In line with previous findings, it was possible to see the greatest effects and changes in situations where visual sensory cues were activated by manipulating the illumination conditions, and the fish displayed smooth tracking behavior in the light of visual cues [8, 15]. We found that the conductivity factor had a significant impact on tracking performance; however, we determined that the window state did not have the same effect on refuge tracking performance according to tracking error.

Confirming our first hypothesis, different illumination conditions had a major impact on refuge tracking for both species of fish. The fish performed active sensing movements that required more scanning and cost when transitioning from light to dark. Fish exhibited high frequency response gains and low phase delays when tracking in light compared to dark. This is similar to the results shown previously in the literature [8].

Whether the refuges had windows or not had a limited effect on tracking performance, as mentioned in previous studies [8]. According to our second hypothesis, the presence of windows will affect both the electrosensing and vision of the fish. Analysis revealed that this effect made a statistically significant difference only in the black ghost knifefish species. This was not the case for glass knifefish. Further investigations are required to understand why.

As we expected in our fourth hypothesis regarding conductivity, we expected that the tracking performance of fish at low conductivity would be much better than at other conductivity levels. Our results confirmed this. Similar results had been demonstrated in previous studies [8].

When we considered performance similarities and differences between species in our final hypothesis, our ANOVA results revealed a statistically significant difference between species.

We would expect the presence of windows in refuges to have a statistically significant effect on the tracking behavior of both species. Contrary to our expectations, only for black ghost knifefish did the presence or absence of windows in the refuges create a statistically significant difference in the results. However, we did not observe a significant difference for glass knifefish.

Refuge length had the greatest effect on tracking performance. According to our analysis results, the most important factor affecting the tracking performance was the length of the refuge. In general across these two weakly electric fish species, shorter refuges exhibited higher gain, whereas longer refuges caused greater phase delay under similar conditions.

There was a statistically significant difference between tracking performances in light and dark conditions. We observed a notable degradation in the fish's ability to track the refuge with the transition from a light to a dark illumination condition. When comparing tracking performance in light conditions with tracking performance in dark conditions, the fish exhibited increased frequency response gains and reduced phase delays. Specifically, an overall increase in phase delay was observed in dark conditions, particularly at higher frequencies. Also, based on both RMSE values and tracking error, the highest refuge-tracking error were in dark conditions.

Fish movements increased with increasing conductivity. There were differences between the movements of fish in the dark and in the light. In dark condition, fish moved their bodies more and make active sensing movements such as tail bending. These active sensing movements helped to shape electrosensory feedback [6]. The increase in fish movement with increased conductivity could be explained by the fish trying to adapt spatio-temporal patterns of neural activity as well as low conductivity.

The analysis results revealed that while the presence of windows becomes important in the absence of light, the presence of windows in the refuges may not make a significant difference in the presence of light. In a dark and dimlight environment, we would expect the presence or absence of windows to be statistically significant under a condition where weakly electric fish rely more on information from the electrosense rather than their eyesight. Our results confirmed this expectation. On the other hand, the presence or absence of windows would not make a statistically significant difference in a scenario where the fish weights the information from the sense of sight more in light conditions.

Individuals of one species exhibited better tracking performance at lower water conductivities. The observation of better sensing performance (longer sensing distances and lower miss rates) in black ghost knifefish at lower water conductivities may be explained by the fact that there is less attenuation in the electric field, resulting in a wider sensing range, and the electroreceptors are better tuned to electric field properties under low conductivity conditions. The change in conductivity was not clearly observed in glass knifefish. This can be explained by the fact that although the two species share some similarities in their electrosensory systems, they had some anatomical and physical differences that play a role in their electrical communication and navigation abilities. These findings in our analysis were in agreement with the literature study that observed that the conductivity range of black ghost knifefish water varies with the sensing distance, and the best performance was at low water conductivity and the lowest performance at high conductivity [14].

Species-specific anatomical differences may influence visual and electrosense weighting. In explaining the differences in refuge tracking performance between black ghost knifefish and glass knifefish, we could infer that although both species have electrosensory systems, there may be some differences between them in terms of electrical organ discharges (EODs) and electrocommunication behavior [52]. Differences in organs of vision between species may explain why the presence of windows in refuge walls made a significant difference only for black ghost knifefish species. For the black ghost knifefish with poor eyesight, it can be giving weight to its electrosenses.

4.6. Limitations

We expected significant differences in conductivity, illumination condition, and refuge structure in both fish species. Although these differences occurred in both black ghost knifefish and glass knifefish, the presence or absence of windows in the refuges revealed statistically significant differences only in black ghost knifefish. One of the reasons for this may be the insufficient number of samples or the differences in the anatomical features of the fish.

The higher the conductivity, the greater the conductivity in the water. The electric field sent by the fish returns to the receptors in much shorter paths. Thus, the range of the electrosensory system decreases. Therefore, we expect tracking performance to deteriorate as conductivity increases. Results were generally much better at lower conductivities [22, 53]. Tracking error were much less.

The presence of windows in the refuge structure affects both the fish’s electrosense and its vision, especially in light illumination conditions. A window with a homogeneous distribution can be replaced with window vary in a width and spacing with a heterogeneous distribution to see how it will affect the experimental results [8].

When examining the effects of refuge lengths and comparing individuals belonging to different species, the problem of whether the effect was caused by the physiological structure of the fish or other factors may be resolved by subjecting fish of the same size to experiments.

5. CONCLUSION and FUTURE WORKS

In this thesis study, we investigated the effects of sensory salience on tracking performance in two different weakly electric fish species. For this, we carried out experiments on the manipulations of 4 environmental conditions: conductivity, illumination, windows and length.

According to the experimental procedure we created in the experimental setup we developed, we conducted experiments with 5 individual fish for each of the weakly electric fish species, black ghost knifefish and glass knifefish.

We used two different metrics to measure the fish's refugee tracking performance. One of them was RMSE and the other was the tracking error. We interpreted the findings we obtained with mixed design five-factor ANOVA.

Our results, consistent with previous studies, revealed that tracking performance gradually deteriorated when transitioning from light to dark under illumination conditions [15]. Highest tracking error values according to both RMSE values and tracking error were in dark conditions.

It is a robust finding that both refuge length and illumination had a major impact on refuge tracking behavior, in line with previous studies [8]. In line with our expectations, both fish species exhibited the best tracking performance at low conductivity. Whether the refuges had windows or not made a statistically significant difference only for black ghost knifefish. There was a statistically significant difference between species in terms of tracking performance.

The presence of windows opened on the refuges in well illuminated conditions had a minor effect on the tracking performance, while the presence of windows on the refuges in dark conditions positively affected the tracking performance and makes a big difference in the results.

Species-specific anatomical differences may influence visual and electrosense weighting (Black Ghost Knifefish has relatively poor eyesight to Glass Knifefish).

A more comprehensive study could be conducted on the effects of windows within the refuge. Further behavioral experiments are required to investigate the electrosense and vision differences between Black Ghost Knifefish and Glass Knifefish.

This study provided the groundwork for comparative system identification and mathematical modeling studies to be planned in the future. Comparative analyzes will be performed using a system identification approach for refuge tracking performance with the inclusion of *Apteronotus leptorhynchus* in future studies.

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