# THE NEURAL CORRELATES OF MIGRATION IN FISH


**By**

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**Research Report Submitted to the Faculty of Health Sciences, the University of the Witwatersrand in fulfilment of the degree Master of Science in Medicine**

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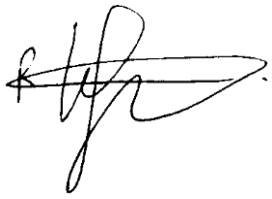
**12 AUGUST 2022**

**DECLARATION**

I, Rabelani Negota, declare that this research report is my original unassisted work. I am submitting this work for the Master of Science in Medicine degree at the University of the Witwatersrand, Parktown, Johannesburg, South Africa. The research report has not been submitted before for any degree or examination at any other University.



(Candidate Signature)

I declare this on the 12th day of August 2022 in Johannesburg, South Africa.

**DEDICATION**

I dedicate this research work to my heavenly Father for His grace, wisdom and strength to accomplish this task.

**PRESENTATIONS ARISING FROM THIS STUDY**

1. Poster: The Neural correlates of migration in fish. Authors: R. Negota, P. Cowley, P, Manger, A, Ngwenya. Presented at the 15th SONA/4th GNS hybrid conference, Ghana, 2021.
2. Poster: The Neural correlates of migration in fish. Authors: R. Negota, P. Cowley, P, Manger, A, Ngwenya. Presented at the African Inclusive Microscopy: Student Microscopy Symposium 2021.

**ABSTRACT**

The hippocampal pallium and its homologues show remarkable plasticity, including volumetric changes and changes to cell density and even cell morphology in response to changes in the external environment. Across vertebrates, this brain region has been involved in navigation and spatial memory. Many studies have revealed specialisations associated with large home ranges, environmental complexity, and migratory behaviour in birds, rodents, and mammals. Much research has been done on fish movement patterns as it relates to migratory activity, as this is of significant ecological and economic importance. However, studies of the neural correlates of fish migration are few. The study aimed to identify potential specialisation of the fish pallium for migratory behaviour.

This project compared the dorsolateral pallium of resident fish species (*Pometopon grande* and *Diplodus capensis*) and migrant fish species (*Pomotomus saltatrix* and *Lichia amia*). Fish were procured through donations from local anglers on the coast of KwaZulu-Natal and Eastern-Cape, South Africa, and approximately three brains of each species for analysis. For each species, studies of neuronal morphology, using Golgi impregnation technique, and the volume of pallial nuclei was made (from Nissl-stained sections). Specifically, neurons of the dorsomedial (a homologue of the amygdala) and dorsocentral (a homologue of the isocortex) were made to identify potential specialisations of dorsolateral pallium neurons.

Generally, dorsolateral and dorsomedial neurons are similar, with dorsocentral neurons being remarkably different in the volume of dendrites. However, neuron morphology shows no correlates of migratory behaviour. However, differences in the relative proportion of the different pallial nuclei imply some adaptation in migrants, including overall reduced brain size and selective enlargement of the dorsolateral pallium in migrants. Overall, our findings suggest that migratory fish may be relying more on responding to specific cues for navigation during migration instead of spatial memory centred in dorsolateral pallium. Anatomical areas associated with sensory cue perception could potentially show more adaptation for migratory behaviour.

**ACKNOWLEDGEMENTS**

A special thanks to my heavenly Father, God Almighty, for granting me the grace, strength and wisdom to accomplish this research work.

I am grateful to my parents, Mr Harold Negota and Mrs Elisa Negota, for their support throughout this Master's journey, Mr Victor Negota and Mrs Beauty Negota for providing an enabling environment staying with them during my write-up.

A special appreciation to my supervisor, Dr Ayanda Ngwenya, for her prompt feedback every time I submit a draft. I am grateful for the outstanding support and guidance throughout this process. I could not have asked for more.

I would like to thank the research group from SAIAB led by Professor Paul Cowley for collaborating with us and assistance in fishing. A special thanks to local anglers in Margate and Port Alfred for their assistance with fishing and donations.

Also, I would like to thank the support of NRF and the school of Anatomy (Wits) for financial support in funding travelling, laboratory equipment and consumables.

Finally, I wish to thank my friends Godwin Kalu, Larbi Yaw-Akwu, and Dr Illke Malungu for yor peer review of this week. Dr Joao Coimbra and Ntando Ndinisa for assistance with labouratory work. Bongiwe Majezi, Meck Nyanda, and Nondumiso Mabaso your encouragement and social support made this journey much easier.

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## LIST OF ABBREVIATIONS

**DC** Diplodus capensis

**Dc** Dorsocentral

**Dd** Dorsodorsal

**Dl** Dorsolateral

**Dm** Dorsomedial

**Dp** Dorsoposterior

**LA** Lichia amia

**OB** Olfactory bulbs

**PG** Pachymetopon grande

**SAIAB** South African Institute for Aquatic Biodiversity

**PS** Pomatomus saltatrix

**V** Ventral

**WM** White Matter

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## CHAPTER 1: INTRODUCTION

Animal migration is defined as a directed movement from one habitat to another by a population or species, often prompted by the inability of their primary habitat to meet all their needs (e.g. changes in environmental temperature, food availability, and a spawning or reproduction habitat) (Binder et al., 2011). The process generally follows periodicity; it can occur on a daily basis, as seen in pelagic lake dwelling coregonid fish (Mehner & Kasprzak, 2011) or seasonally, as in Kokanee salmon (Brönmark et al., 2013). Long-distance migration, in particular, is believed to have considerable effects on the brain since it is both physiologically stressful and cognitively demanding.

The mammalian hippocampus and its homologues in non-mammalian vertebrates is involved in learning and memory (Dhikav & Anand, 2012), as well as spatial navigation (Stella et al., 2012). The hippocampus has been studied extensively and shows significant structural and functional changes associated with the external environment, including changes in volume, neuronal density and neuronal morphology (Sherry et al., 1992; Maguire et al., 2000; Kulkarni & Firestein, 2012). These structural and functional changes in the hippocampus can be deleterious or advantageous to the individual.

The stress response has a significant influence on hippocampal structure and function because the hippocampus has an abundance of adrenal steroid receptors (McEwen, 2002), such as mineralocorticoids (type I) and glucocorticoids (type II), to which stress hormones bind with high affinity (Kloet & Marian, 1996). In general, chronic stress has deleterious effects on the hippocampus, resulting in the retraction of dendrites, as well as reduction in dendritic branching in the neurons found in the hippocampus, especially CA3 pyramidal neurons (Watanabe et al., 1992). CA1 and dentate gyrus granule neurons are assumed to be similarly affected (Watanabe et al., 1992). The amygdala, like the hippocampus, also mitigates stress, and their basal dendrites were similarly retracted by chronic stress (Watanabe et al., 1992).

Conversely, spatial navigation is associated with increases in hippocampal volume as seen in food caching birds; where birds that cache their food in their environment during favourable seasonal conditions in order to locate it when conditions are unfavourable have relatively larger hippocampi than closely-related species that do not (Sherry et al., 1992). The same is seen in voles and deer mice that travel long distances in search of a mating partner, the males of the

species have a relatively greater hippocampal volume than females which do not travel to find mates (Sherry et al., 1992). This phenomenon has also been reported in humans; Maguire et al., (2000) reported a significantly larger hippocampal volume in licensed London taxi drivers when compared to people who did not drive taxis. The author also observed a positive correlation with regards to hippocampal volume and increasing years of experience.

From the studies above, we observe that in birds and mammals, extensive spatial navigation skills are correlated with neural specialisations, such as relatively larger hippocampal volume. It would be interesting to see if the same principle applies to migrating fish compared to resident fish, particularly if specialisations extend to neuronal morphology.

Mammals and birds show greater cognitive ability than fish, nevertheless, the fish brain is similarly organized, with many brain structures homologous to those of other vertebrates. The dorsolateral pallium of fish has been proposed as a structural homologue of the mammalian hippocampus, whereas its dorsomedial pallium is a homologue of the mammalian amygdala (Vargas et al., 2009). The dorsolateral pallium is therefore believed to be involved in spatial learning and is implicated in the temporal aspect of learning (Vargas et al., 2009). The dorsomedial pallium plays a role in learning emotional traces and avoidance learning (Vargas et al., 2009). Additionally, the fish brain shows similar specialisations associated with spatial memory, female blennid fish show relatively larger hippocampal pallium than males (with a smaller home range) (Costa et al., 2011).

Approximately 2.5% of all fish species undertake migration, with some travelling over considerable distances (Brönmark et al., 2013). The most notable example is the migration of Pacific salmon that migrate in order to spawn before dying (known as semelparity) (Binder, 2011). But, migration also often occurs seasonally, with some species undertaking several migrations throughout their lifetime (known as iteroparity) (Brönmark et al., 2013).

Migration is both a cognitive process as well as a stressful one. It is energetically demanding, yet feeding is rare during migration (Binder, 2011). There are also significant morphological and behavioural changes that occur during migration, such as atrophy of the alimentary canal and digestive system (Binder et al., 2011). Pacific salmon experience a reduction in brain volume in addition to significant changes in brain morphology, for example, neurodegenerative markers that are similar to those observed as a result of age-related neurodegeneration have been seen in some semelparous species (Maldonado et al., 2002). These impacts on brain morphology and volume in these species are believed to be associated with chronically high

levels of glucocorticoids, inhibition of neurogenesis, a rise in glutamate levels or cortisol releasing factor (CRF) (Bremner, 2002).

Given the competing stimuli which affects neurons of the dorsolateral pallium of migrating fish such as increased learning and spatial memory, which tend to incite increased neurogenesis and dendritic branching, and conversely, chronic stress which tends to incite reduced neurogenesis and dendritic branching it is interesting to identify if these neurons show particular specialisations for migratory behaviour displayed by migratory species. Fish having higher levels of neuroplasticity in the adult dorsolateral pallium than adult human hippocampal pallium makes this study even more interesting considering that migrant birds, rodents (Sherry et al., 1992) and London taxi drivers (Maguire et al., 2000) show specialisations for migratory behaviour than closely related resident species. Additionally, understanding neural correlates of migratory behaviour equips our management of fisheries as a crucial economic and cultural resource in South Africa and the environment thereof to which fish contribute biodiversity.

# CHAPTER 2: LITERATURE REVIEW

Several lines of evidence exist showing how species' behaviours that can be described as an adaptive response to their environmental needs have an impact on brain and neuronal morphology.

Much research has been done on the neural structures associated with specialised sensory systems, for example, mormyrid fishes which have a highly specialised and well developed electrosensory system are known to have a 'gigantocerebellum' that is thought to be primarily involved in electroreception (Meek et al., 2008; Sukhum et al., 2018). Similarly, specialised behaviour(s) are known to also correlate with specific neural structures.

Migration can be viewed as one such adaptive behaviour, particularly because this behaviour is not commonly seen throughout the animal kingdom. In studies of birds and mammals, hippocampal volume and neuronal morphology are known to vary (even between closely related species) depending on the species' reliance on spatial memory (Sherry et al., 1992).

The hippocampal region of birds and mammals has also been shown to undergo significant plastic changes as the external environment changes. For example, the rate of neurogenesis and hippocampal volume overall have been shown to change with reproductive status in Richardson's ground squirrels (Burger et al., 2014). Similarly, plastic changes have been observed in the hippocampus of these individuals as a result of hibernation (Brinkman, 2019). These changes may not necessarily be indicative of specialisation of this region, however, they demonstrate the effect that external conditions can have on cell morphology. Numerous studies in animal models on the effects of chronic stress and environmental enrichment on hippocampal neuronal morphology provide support for these observations made in the wild.

In the context of long-distance migration, it is of interest to understand the interaction between the cognitive demand associated with navigation and the maintenance of spatial memory and the physiological stress associated with migration.

## 2.1. Neuroplasticity

Neuroplasticity is a non-pathologically change in the brain morphology often resulting in brain function efficiency (Kolb & Whishaw, 1998). Neuroplasticity occurs through sensory integration, neural circuits, and topographical maps, and occurs as a response to various stimuli

from the environment. Stimuli integration allows for appropriate responses to input in the brain (Kolb & Whishaw, 1998). As inputs and outputs are recorded, neural circuits are formed, resulting in topographical maps (Cang & Feldheim, 2013). In other words, topographical maps bring about the integration of sensory modalities and, thus, sensorimotor responses. For example, when infants learn a language, their brain initially has broad tonotopic maps and gradually a refined tonotopic map and language follows (White et al., 2013). The refinement of neural circuits occurs concurrently with morphological changes in neurons (Kolb & Whishaw, 1998). Likewise, migrant fish with advanced spatial skills are thought to have refined neuronal circuits responding to sensory cues and developing accurate navigational strategies.

Structural plasticity in the brain involves modifying neural tissue, including brain size, dendritic branching, spine density, soma and dendritic volume (Bozelos & Poirazi, 2017). Neuronal changes are visualised through immunohistochemistry, microscopy, and stereology as these tools translate to tangible and measurable quantities (Kolb & Whishaw, 1998). An expectation with increased morphological changes on neurons is increased metabolic activity supporting these neuronal changes (Kolb & Whishaw, 1998). For example, the increased metabolic activity of dendritic branching and synapse per neuron would be indicated by increased astrocytic material and blood capillaries (Kolb & Whishaw, 1998). More importantly, morphological changes are associated with behavioural differences in living organisms (Kolb & Whishaw, 1998).

### 2.2. Behavioural Links to Neuroplasticity

Several studies have shown the association between structural plasticity and specific behaviours in which individuals are specialised. Like humans, birds show comparable intelligence and tool use which have been associated with structural modifications in their brains (Olkowicz et al., 2016). Even though birds generally have a smaller absolute brain volume, corvids, which are regarded as intelligent, display a high density of neurons comparable to mammals (Olkowicz et al., 2016). One of these high-density areas in corvids and parrots is the pallial telencephalon (Olkowicz et al., 2016). Corvids also resemble mammals as tool users, and therefore display a relatively larger nidopallium (homologue of mammalian pre-frontal cortex) than non-tool users (Cabrera-álvarez & Clayton, 2020). These

selective increases in brain volume are believed to enable corvids and parrots to be more adept with problem-solving (Cabrera-álvarez & Clayton, 2020).

Structural plasticity tends to make individuals adapt to their environment, which has evidently been true for fish as well (Gonda et al., 2011). The rearing environment of fish has been shown to impact cerebellar and total brain volume (Kihslinger & Nevitt, 2014). Fish reared in a more naturalistic environment, such as a river, were larger than laboratory-grown fish, and fish reared in rocky pools had a larger total brain volume than lab-grown fish (Kihslinger & Nevitt, 2014). Incorporating rocks in fish tanks of laboratory fish led to the observation of cerebellar volume similar to river-reared fish than fish reared in simple tanks without rock landmarks (Kihslinger & Nevitt, 2014). The findings suggest that a complex or enriched environment allowed for increased cues received and processed by the fish, leading to neuroplasticity. Furthermore, White & Brown (2015) suggested that fish from rocky pools were evolutionarily required to remember their spatial environment more and gain spatial skills to survive in their environment than fish in low tide sandy regions. These studies depict the brain as malleable rather than a rigid structure.

Literature has also highlighted that apart from the whole brain, the brain area specialised for specific behaviours display neuronal changes (Triki et al., 2019). Social cleaner fish (*Lambroids dimidiatus*) from high population density reefs are more socially competent than cleaners from low population density, their social competence has been positively correlated with a larger forebrain (including diencephalon and telencephalon) (Triki et al., 2019). These findings supported the social brain hypothesis, i.e. active cognitive integration and social complexity areas can drive neuronal changes in specific brain regions facilitating those roles (Triki et al., 2019). Similarly, the hippocampal pallium (which is known to be relatively larger in homing pigeons and food-caching birds) is relatively larger in female blennind fish with a larger home range, than in males (Costa et al., 2011).

Studies above demonstrate a clear association between high stimulation such as spatial cues and specific adaptions in the brain (Kihslinger & Nevitt, 2014). Differences in environment (Kihslinger & Nevitt, 2014), sex, homing and migratory behaviour (Costa et al., 2011) were also associated with differences in the structural changes in the brain.

## 2.3. The Hippocampus and its Modulation by External Factors

### 2.3.1 Fish brain and its divisions

Fish are considered to have more malleable brains than mammals (Zupanc, 2008). The teleost fish has a higher rate of adult neurogenesis than mammals, moreover, the fish brain is known to show a high rate of regeneration following injury (Zupanc, 2008). The fish brain has been described as lacking complexity (Zupanc, 2008) although consisting of homologous structures of a mammalian brain. While the dorsolateral pallium has been proposed as a structural homologue of mammalian hippocampus, and dorsomedial as the homologue of the amygdala (Broglio et al., 2003; Vargas et al., 2009), the dorsocentral pallium is regarded as an area of integration and modulation of these superficial pallial regions (Demski, 2013).

### 2.3.2 External factors modulating the hippocampus

Hippocampal neurons are vulnerable to external environmental changes (Ebbesson & Braithwaite, 2012) as observed in breeding and migratory activity of birds and rodents (Brinkman, 2019; Sherry et al., 1992). Brinkman (2019) found that spine density changes can occur based on season in seasonally reproducing Richardson's ground squirrels. Breeding season was marked by reduced spine density, as a result of high stress levels associated with competing with males for mating (Brinkman, 2019; Michener, 2015) and seasonal effects of photoperiod seasonal changes whereas in hibernation, these animals recovered (Brinkman, 2019). Barkan et al., (2014) found that migrant reed warblers had increased neurogenesis in the hippocampus and nidopallium in migratory season compared to resident clamorous warbler. And when both species had fewer new neurons during breeding season, the authors proposed that increased neurogenesis in migrants during migratory season was related to increased need for spatial navigation (Barkan et al., 2014). Migratory behaviour presents a complex case with an increased need for cognition, spatial navigation, adaptation to environmental changes, and stress.

Stress is known to cause considerable structural changes in hippocampal neurons (Leuner & Shors, 2013). Studies like Watanable et al., (1992) and Magarinos et al., (1996) reported reversible shortening and atrophy of apical dendrites in the hippocampus and basal amygdala dendrites in mammals associated with acute stress. Chronic stress causes irreversible atrophy (Watanabe et al., 1992b). In mammals, hippocampal and amygdala neurons are negatively

affected by stress because they mitigate stress by possessing many adrenal steroid receptors such as glucocorticoids and mineralocorticoids (Kloet & Marian, 1996).

There is a view in literature pointing to the benefits of acute increases in cortisol in fish and mammals, particularly as it relates to spatial memory and cognition (Carruth et al., 2002). For example, rats performed better on an eight-arm maze test after acute constraint stress (Luine et al., 1996). In Kokanee salmon (*Oncorhynchus nerka kennerlyi*) and Pacific salmon (*Oncorhynchus*), cortisol is thought to ensure reproductive success as these species migrate to spawn in their natal streams (Carruth et al., 2002). Carruth et al., (2002) suggested that cortisol facilitates the imprinting of their natal stream, which helps the fish navigate during migration.

Migratory fish (Carruth et al., 2002) and birds (Liu & Swanson, 2014) have been found with high plasma cortisol levels. Elevated plasma cortisol levels are associated with migratory restlessness (increased anxious locomotion behaviour before migration) (Eikenaar et al., 2014; process Sudo & Tsukamoto, 2015), as well as other physiological changes such as reduced egg production in preparation for migration (Brönmark et al., 2013). During the migratory, individuals additionally face food deprivation, few to no stops along the route, increased predator interactions (Bronmark et al., 2008), and altered ambient conditions such as oxygen and nutrient availability (Chapman & Hulth, 2012), all of which can be seen as additional stressors.

Notably, Kokanee and Pacific salmon die approximately two weeks after the spawn migration, and have been reported to show reduced brain volume and senile plaques resembling those found in human Alzheimer's disease and ageing following migration (Maldonado et al., 2002). The death of this species is correlated with increased cortisol levels and reaching sexual maturation (Maldonado et al., 2002). However, it is also speculated that their death could be programmed (Carruth et al., 2002). Lastly, Brönmark et al., (2013) remarked that the difference between semelparous Pacific salmon and iteroparous fish species is that iteroparous species were more efficient energy users by not using all their energy for reproduction and migration. It could be possible that iteroparous fish show neural specialisations for migration as well.

### 2.4. Migratory Behaviour in Fish

Fish migration is an essential part of ecology and is crucial for migrants and non-migrants' survival (Kokko & López-Sepulcre, 2006). About 2.5% of all fish species are estimated to migrate (Binder et al., 2011). However, the range of distance in migration is variable; eels

(*Anguilla*) and tunas (*Thunnus*), for example, can migrate thousands of kilometers across the Atlantic and Pacific oceans respectively; whereas local species such as shad (*Pomatomus saltatrix*) and garrick (*Lichia amia)* have been recorded to have migrated 1760km (Hedger et al., 2010) and 825km (Murray et al., 2017) respectively. Fish in estuaries are impacted by tide and photoperiod for example in Shad (Maggs & Cowley, 2016). Therefore, fish would rather flow with the current than against it even during in-shore or off-shore movement (Hudson, 2019) with the exception of pacific salmon, which have been observed swimming against the current (Taylor, 2022).

### 2.4.1. Fish navigation in the open water

Studies have suggested that fish use the sun for orientation in open water, particularly the angle of the sun in horizontal plane (Binder et al., 2011). However, in the absence of the sun fish have been found to pick up on polarized light (Binder et al., 2011). Olfactory cues are also instrumental for orienting homing salmon species that imprint the odour of the stream they were born in and thereafter followed to find the natal stream (Binder et al., 2011). Carruth et al., (2002) mentions that cortisol facilitates imprinting of natal streams. Additionally, geomagnetic cues are thought to orient fish, turtles and seabirds (Luschi, 2013).

### 2.4.2 Brain activity during migration or spatial navigation

When navigating an area, an organism is guided by two cues, idiothetic/egocentric from proprioception including vestibular feedback, and allothetic/allocentric, from landmarks in an environment (Solari & Hangya, 2018). These cues help create cognitive maps of the environment, the egocentric codes the route navigation, and the allocentric creates a world view or grids of a particular space (Solari & Hangya, 2018).

Teleost fish have been found to use various spatial navigation strategies including multisensory cues such as the sun, olfaction, geomagnetic maps (Binder et al., 2011; Carruth et al., 2002) and electrosensory information as in the case of Gymnotiform fish (Fotowat et al., 2019). In addition, fish also use map-like spatial memories similar to what is seen in mammals and birds (Quintero et al., 2021). Thus, making fish dynamic in their spatial navigation. Mammalian studies guided most of what we know concerning spatial navigation strategies. However, teleosts have equally been shown to be spatially accurate (Perera et al., 2016). Mammalian studies include the discovery of place cells by O'keefe & Dostrovsky (1971), head direction cells by Taube et al., (1990), and grid cells by Hafting et al., (2005). Additionally, the

conjunctive grid-head direction cells (Sargolini et al., 2006), border cells (Solstad et al., 2008), and, more recently, speed cells (Kropff et al., 2015) have been instrumental to our understanding of spatial navigation in mammals. Although analogous cells have yet to be identified in the fish brain, ablation studies, show that fish have similar deficits as mammals. Additionally, a recent study in goldfish identified cells responsive to the edge of the environment, head direction, as well as swimming velocity (Vinepinsky et al., 2019, 2020), thus, teleost navigation may include similar cells to those described in mammals.

The hippocampal pallium creates allocentric cognitive maps that guide navigation and portray a global view of spaces in mammals (Schiller et al., 2015) and fish (Quintero et al., 2021). In teleosts egocentric cue perception is thought to be centred in the optic tectum (Quintero et al., 2021). The interconnected nature of the optic tectum makes this area suitable for integration of sensory modalities and sensorimotor translation as it relates to body-centred orientation (Quintero et al., 2021). In relation to allocentric cue formation in the hippocampus, place cells code the environment so that a spatial memory of the animal in that location occurs (Olafsdottir et al., 2017). Place cells fire in a sequence, and even during sleep, the same pattern occurs to store the spatial memory during sleep and wakefulness (Olafsdottir et al., 2017). Dorsodorsal pallium cells (DDi) described in Gymnotiform fish are thought to function like mammalian place cells and border cells, firing with respect to particular locations (Fotowat et al., 2019). DDi cells differ from place cells as they fire at multiple locations, and DDi cells are unlike border cells due to firing at all borders (Fotowat et al., 2019). DDi cells have only been identified to function like place cells in electrosensory fish like the Gymnotiform fish, thus, may not apply to teleosts selected in this study. Rather there is overwhelming evidence for dorsolateral pallium neurons working in map-like spatial memory formation.

There remains some unknown information about spatial navigation in fish, including the morphological differences of brain regions involved in spatial navigation between migrant and resident species.

### 2.4.3 Significance of investigating correlates of migration in fish

Fish are found in all bodies of water; more than 34300 fish species are estimated to be present worldwide (Fishbase, 2020), of these species, teleosts make up 95% (Nelson, 1994). Additionally, 2.5% of fish species are known to go through migration (Binder et al., 2011) which is important for the ecosystem and economy. Overfishing, therefore, threatens the proper functioning of the entire ecosystem (Barange et al., 2018). Moreover, fish are of recreational

and cultural importance and fisheries play a large role in the global economy. In 2018, the fishing industry provided produce worth US$263.6 billion, thus, providing jobs to over 59.5 million people by primary fish farmers and aquaculture worldwide (FAO, 2018).

Understanding fish migration is crucial as it adds to the understanding of the evolution of brains, precisely why some fish evolved to migrate, and various pressures they overcame (Chapman & Hulth, 2012). Additionally, this understanding potentially adds to efforts to manage fish stocks as a resource in the ecosystem. Given that the environment critically influences fish migration, migration occurs as an adaptive response to changes in their habitat (Binder et al., 2011), fish migration already faces threats from human activities such as building dams or infrastructures in the path of migrating fish, pollution in rivers, dumping toxicants, and climate change. These human activities result in changing water temperatures and further contribute to the depletion of fish stocks, a renewable food source in communities (Binder et al., 2011). Therefore, any effort towards understanding fish migration which includes adding restrictive measures in fishing, protects the same environment and ecosystems sustaining all other living organisms.

Finally, to better understand the evolution of the human brain, more efforts are needed to understand the brain structures of other animals besides mammalian species (Manger et al., 2008). Our understanding of neuroplasticity and spatial navigation could vastly be improved by studying these concepts in fish because fish make up 47% of vertebrate animals (Manger et al., 2008), and neuroplasticity in adult fish occurs at a higher rate than in mammals (Zupanc, 2008).

## CHAPTER 3: RESEARCH AIMS AND OBJECTIVES

### 3.1 Aim

The study aimed to compare the dorsolateral pallium (a homologue of the mammalian hippocampus) in migratory and resident species of fish to identify potential specialisation of the dorsolateral pallium for migratory behaviour.

### 3.2 Objectives

1. To quantify the volume, neuronal dendritic fields, and dendritic morphology of lateral pallium neurons in migratory and resident fish species
2. To determine differences in volume and dendritic morphology in the dorsolateral (Dl), dorsomedial (Dm) and dorsocentral (Dc) pallium in migratory species.
3. To establish neural correlates of migratory behaviour**.**

### 3.3 Rationale of the Study

Many studies have revealed specialisations associated with large home ranges, environmental complexity (Kihslinger & Nevitt, 2014), and migratory behaviour in birds, rodents (Sherry et al., 1992), and mammals (Maguire et al., 2000). Much research has been done on fish movement patterns as it relates to migratory activity in southern Africa (Maggs & Cowley, 2016), as this is of significant ecological and economic importance (Kokko & López-Sepulcre, 2006). However, studies of the neural correlates of fish migration are few, despite the 2.5% of 34800 species of fish which migrate (Binder et al., 2011). Lastly, there is an interesting migratory behaviour of Kokanee and Pacific salmon only migrating once in their lifetime and dying approximately two weeks after a spawn migration, and have been reported to show reduced brain volume and senile plaques such as those found in human Alzheimer's disease and ageing whereas Atlantic salmon and other fish species migrate annually (Maldonado et al., 2002).

# CHAPTER 4: METHODOLOGY

## 4.1 Ethical Consideration

All animals were treated and used according to the University of the Witwatersrand Animal Research Ethics Committee (AREC) guidelines, which parallel those of the NIH for the care and use of animals in scientific experimentation (Clearance number AESC19 05 003).

## 4.2 Study Collection

A review of the fish movement studies in South Africa conducted by Maggs (2017) comprising fish movement data collected over the past nine decades was used to determine the most suitable fish for study. In this study, a total of 101 publications were surveyed and a number of methods were used to verify the movement of species (Maggs, 2017). The selected species of interest for the current study in the residents' classification were blacktail (*Diplodus capensis*) and bronze bream (*Pachymetopon grande*). The migrant species selected for this study were shad (*Pomatomus saltatrix*) and leervis/garrick (*Lichia amia*). Fish were collected through dedicated sampling trips in the coastal regions of South Africa and donations from local anglers.

## 4.3 Study Species

A total of eleven individuals from species with movement patterns that can be categorised as migratory and non-migratory/residents. Migrants are fish that seasonally relocate between habitats that are spatially separated to distances between 51-500km or greater than 500km and resident or non-migratory fish are repeatedly fixed in a familiar habitat within a 0-5km distance (Maggs, 2017) classification were caught using conventional fishing methods, as well as donated by recreational anglers. There was no preference for either male or female migrant specimens as Maggs (2017) showed no preferences for either sex in the chosen species and it is likely that attempt to control for sex of individuals would be limiting to our data collection. Migrating fish were collected during winter season (June to August) which is a migratory season (Govender, 1996) to accurately deduce any specialisations and impacts of migratory behaviour in the dorsolateral pallium. Breeding begins in the end of winter and early spring to summer months for *Pomatomus saltatrix* (Govender, 1996). Specimens were collected from

Margate, KwaZulu Natal and Port Alfred, Eastern Cape, South Africa between March 2020, and July 2021.

### 4.4 Tissue Processing

After capturing the fish, they were euthanised using a mixture of clove oil and 100% alcohol in a 1:0.1 ratio. The mixture was further dissolved in a bucket containing sea water. Fish were left in this mixture until there was no longer opercular movement (minimum of 10 min). Clove oil has been shown to be effective for immobilising fish and was used as an anaesthetic agent (Sladky et al., 2001). Once specimens were euthanised, total length (TL) body measurements from the head to fork of fish tail were taken, except in cases where only fish heads were donated. Fishes were then transcardially perfused with a cold (4ºC) solution of 0.9% saline to rinse away blood and then transcardially fixed with cold (4ºC) 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB). Following fixation, fish heads were submerged in 4% PFA for 24 hours, then brains were dissected out from the skull. Brains were stored in 4% PFA for approximately three days. Next, brains were weighed and then post-fixed in 0.1% sodium azide in 0.1M phosphate buffer at 4ºC until they were ready for use.

In the case of donated specimens, which had to be within five minutes after the fish was removed from water, fish heads were submerged in a solution of 4% PFA in 0.1M phosphate buffer until the tissue was appropriately preserved, after which the brains were dissected and stored as described above.

One hemisphere of each brain was used for Nissl staining, while the other was used for Golgi impregnation and neuron reconstruction.

### 4.5 Nissl Staining

After post-fixation, hemispheres that were used for Nissl staining were cryoprotected in 30% sucrose in 0.1M PB at 4ºC for 72 hours. Thereafter, the brains were frozen using dry ice and serially sectioned in the sagittal plane at either 50µm or 70µm using a freezing microtome (see table 4.2 for more details). Sections were either sectioned at 50µm thickness or 70µm for optimisation of tissue processing. It was found that 50µm was the most suitable, and thus all subsequent brains specimens were sectioned at 50µm. This has been accounted for in the calculations of pallial volume estimations.

Sections were mounted on 0.5% gel-coated glass slides and allowed to dry. The sections were placed in a 1:1 solution of chloroform and 100% ethanol overnight before they were rehydrated

in a graded series of alcohol and stained for 1 minute in 1% cresyl violet solution. The sections were differentiated in a solution of 70% ethanol containing a few drops of acetic acid before being dehydrated in a graded series of alcohol, cleared in xylene, and cover slipped with DPX. Nissl staining of neurons allowed the identification and mapping of the various regions and nuclei in the brain.

### 4.6 Golgi Impregnation

After Nissl staining, appropriate neurons were localized and impregnated with Golgi stain. To study neuron morphology, the Golgi technique used followed that of Scheibel and Scheibel (1978). On the first day, brain hemispheres were placed in an osmium acid solution made of 1g osmium tetroxide, 8g potassium dichromate crystals, and 300ml of double-distilled water for 48 hours in a dark place at room temperature. These brains were stored in a 150ml brown bottle to avoid interaction with light and allow osmium to stain and fix neurons. Thereafter, these brain hemispheres were placed in silver nitrate solution (0.75%) made up of 2.25g of silver nitrate and 300ml double distilled. Again, the brains were stored in 150ml brown bottles for 24 hours. Tissue processing produced very brittle tissue blocks, and two methods were followed to try and minimize tissue breakage. After that, brains were removed from silver nitrate, and they were ready for embedding. The brain was embedded in 4% low melting agarose after staining to reduce breakage during sectioning, the technique followed was used in Rooks and Garrett (2017). Whereby 4g of agarose was added to 100ml of 0.1M phosphate buffer (Pb), which had been heated up to 50ºC. Agarose and phosphate buffer were left to stir for 20 minutes, after which it was further mixed in a microwave in 40-second increments until it reached boiling point and appeared translucent. The embedded brain tissue was stuck onto the vibratome chuck with 1-2 drops of superglue, the tissue bathed in phosphate buffer. The brain was serially sectioned in the sagittal plane between 70 and 100μm using a vibratome, depending on the thickness appropriate to avoid tissue breakage. Sectioned tissue was placed serially in distilled water to desalinize tissue sections that were sectioned in phosphate buffer. After that, the tissue went through a dehydration series between 30% and 100% alcohol. After which, sections were transferred into a 1:1 solution of methyl salicylate and 100% alcohol, followed by methyl salicylate until they sank to the bottom of the solution. Then the sections were immersed in two xylene solutions for 2-3 minutes, after which they were mounted on 3% gel-coated slides and cover-slipped with DPX. Sections were dried for 24-48 hours in a horizontal position in the dark. The second method involved sectioning tissue blocks (which were not embedded in agarose) in 100% alcohol then allowed to clear in methyl salicylate until

the sections sunk to the bottom and in xylene for 2 minutes. Finally, these sections were mounted on 3% gel-coated slides and cover-slipped with DPX.

### 4.7 Golgi Neuron Reconstruction

Three dimensional virtual slides of regions with appropriately impregnated neurons were taken using the virtual tissue function of Neurolucida software MicroBrightfield (MBF) (Colchester, Vermont, USA) system with a three-plane motorised stage, Zeiss.Z2 Vario Axioimager. The objective of the microscope was at 40X. Neurolucida 360 software MicroBrightfeild (Colchester, Vermont, USA) was then used to trace Golgi impregnated neurons. The first few sections in the most lateral part of the telencephalon and peripheral neurons were used to extract and retrace lateral neurons. Central neurons found in the middle sections, and laterally positioned neurons in the most medial sections were used respectively to retrace central and medial neurons (Figure 5.4). Table 4.1 shows the number of Golgi-stained neurons reconstructed for each individual. A total of 214 reconstructed neurons were used to analyse the dendritic morphology, including dendritic complexity, dendritic length, soma volume, and highest branch order. A total of 165 reconstructed neurons were used to analyse spine density, these neurons were chosen based on the presence and full impregnation of their dendritic spines. Generally, neurons were chosen based on impregnation quality, neurons with incomplete or broken dendrites, superimposed, and undistinguishable from other neurons were not reconstructed.

**Table 4.1:** Neurolucida traced Golgi-stained neuron distribution among migrant and resident species. A total of 214 neurons were used for morphological analysis of neurons from the dorsocentral (Dc), dorsomedial (Dm), and Dorsolateral (Dl) pallium. A total of 165 neurons were used for spine density analysis.

| **Fish ID (n=11)** | **Number of neurons traced** | **Neurons selected for spine density** |
|---|---|---|
| **Resident (n=6)** | | |
| *Pachymetopon grande* (PG1203001) | 15 | 15 |
| *Pachymetopon grande* (PG1203002) | 23 | 12 |
| *Pachymetopon grande* (PG2103320) | 14 | 12 |
| *Diplodus capensis* (DC265) | 35 | 15 |
| *Diplodus capensis* (DC260624) | 26 | 18 |
| *Diplodus capensis* (DC270623) | 15 | 15 |
| **Migrant (n=5)** | | |
| *Pomatomus saltatrix* (PS374) | 20 | 16 |
| *Pomatomus saltatrix* (PS2706A) | 15 | 15 |
| *Pomatomus saltatrix* (PS2706E) | 19 | 16 |
| *Pomatomus saltatrix* (PS280621C) | 16 | 16 |
| *Lichia Amia* (LA2806) | 16 | 15 |

### 4.7.1 Length and volume of cell bodies and dendrites

The fully impregnated neurons were traced using MBF Neurolucida 360 software. Traces of the cell body and dendrites were made throughout the depth of the tissue in the x, y, and z plane. The volume of the cell body was estimated by making at least 3 outlines of the outermost borders of the cell body along the depth of the tissue. The following characteristics of the dendritic tree(s) were calculated, branch order, branch number, branch length, branch volume and complexity.

### 4.7.2 Branch order

The software noted each dendrite's orders and compiled the number of dendrites in each order (as seen in Figure 4.1). The measurement allowed for estimation of the complexity of dendrites in the lateral, medial and central pallium to be examined.

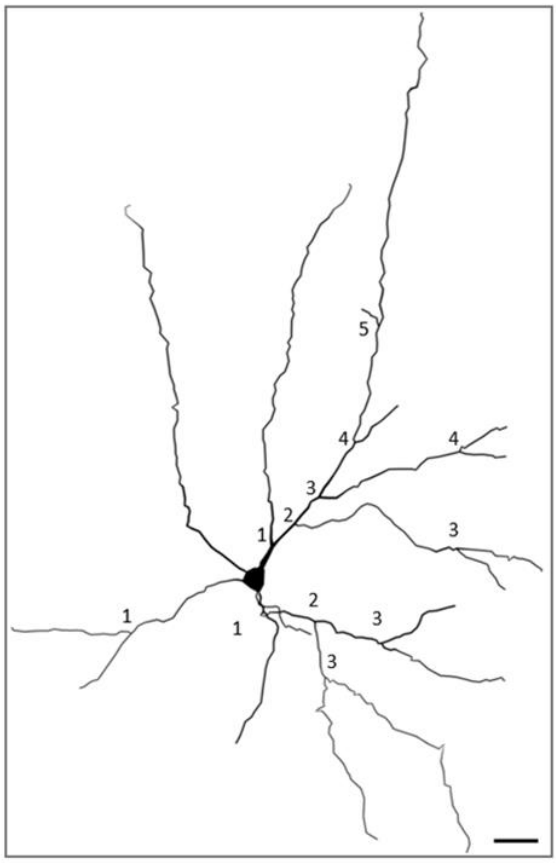



**Figure 4.1.** Two-dimensional Neurolucida tracing displaying dendritic branching. The highest branch order of this magnocellular multipolar neuron is 5, branch orders were used to calculate dendritic complexity index (CI). Neuron was traced from Golgi-stained neural tissue from *Diplodus capensis* in the dorsocentral (Dc) region. Scale bar 25µm.

#### 4.7.3 Complexity index (CI)

Complexity in MBF Neurolucida Explorer is the normalisation and comparison of dendrites among other different neurons. CI is automatically calculated based on morphological data of the neuron, Complexity= [Sum of terminal orders + Number of terminals] * [Total dendritic length ÷ Number of primary dendrites], whereby terminal refers to the order of the terminal ending.

#### 4.7.4 Convex Hull

Convex Hull on MBF Neurolucida Explorer measures the dendritic field, it converts the neuron into a convex polygon, giving the surface area and volume that the neuron occupies in the brain tissue.

#### 4.7.5 Dendritic spine density

Traces of dendritic spines were done using the MBF Neurolucida 360 (MicroBrightField, Williston, Vermont, USA) at 40X magnification. Approximately 15 neurons in each animal were chosen for this analysis comprised of lateral, medial and central pallium neurons. Dendritic spines from the entire neuron were only traced if neurons were stained entirely with visible dendritic spines. Bifurcated spines were taken as single spines. There was no distinction made of spine type and only the spine density per μm was recorded.

### 4.8 Analysis of Pallial Volume

Eight hemispheres were sampled for the estimation of volume for pallial regions in the telencephalon after Nissl staining. Five hemispheres (indicated with * in table 4.2) were used to compare proportional differences of the telencephalon and total brain volume in residents and migrant fish. These five hemispheres were chosen based on availability of scanned whole brain images. Using the Cavalieri probe on MBF Stereoinvestigator (version 11.08.1; 64-bit) software, the boundaries of the dorsolateral, dorsomedial and dorsocentral pallium were contoured based on descriptions from atlases of other species, and the presence of distinct cellular differences and borders; the volume of each region was compared to telencephalon volume, and brain volume. These measurements were compared to the convex hull measurement reporting the dendritic field of neurons in the dorsolateral, dorsomedial, and dorsocentral pallium. A 1 in 4 series of sections was used, with a sample grid size of most sections at 200×200μm and the coefficient error (CE) was not always below 0.1 (Dell et al.,

2015) after all the efforts to consistently keep it below 0.1. This was mostly due to a limited number of countable sections. In addition, the section cut thickness was mostly 50µm and 70µm for one animal. Table 4.2 shows stereological parameters for each animal.

**Table 4.2.** Stereological parameters used for analysis of pallial volume specialisation among migrant and resident species. Neural tissue was section at 50µm and Nissl-stained to delineate the dorsocentral (Dc), dorsomedial (Dm), and dorsolateral (Dl) pallium. The asterisk (*) denotes species that were used to obtain whole brain: telencephalon ratio.

| **Fish ID** | **Sample grid size (µm)** | **Section cut thickness(µm)** |
|---|---|---|
| *Diplodus capensis* (DC258) | 200×200µm | 50µm |
| *Diplodus capensis** (DC270623) | 200×200µm | 50µm |
| *Diplodus capensis** (DC260624) | 200×200µm | 50µm |
| *Pachymetopon grande* (PG275) | 200×200µm | 50µm |
| *Pomatomus saltatrix* (PS2506A) | 300×300µm | 70µm |
| *Pomatomus saltatrix** (PS290631) | 200×200µm | 50µm |
| *Pomatomus saltatrix** (PS2706) | 200×200µm | 50µm |
| *Lichia amia** (LA700) | 200×200µm | 50µm |

## 4.9. Data Analysis

### 4.9.1 Statistical analysis

Past4project 4.05 and STATA version 16 were the tools used to analyse information retrieved from Neurolucida 360 and Neurolucida Explorer. Data retrieved was assessed for normality using the Shapiro Wilk test on PAST 4.05 (Hammer et al., 2001). First, on combined resident and migrant neuron morphology measurements, secondly on residents and migrant groups and thirdly when data was grouped into dorsocentral, dorsomedial, and dorsolateral pallium for each specie. Logistic regression model (generalized linear model) was conducted on

STATA version 16. The clustering effect was accounted for during data analysis and the model was set at 95% confidence level with $p<0.05$ considered as significant.

Kruskal Wallis test was used to determine the differences between relative volume of the telencephalon, the whole brain and different pallial areas of migrants and residents. Likewise, differences between soma volume, dendritic complexity, and spine density of migrants and residents in different pallial areas by Kruskal Wallis. A Dunn's post hoc test was used to analyse magnitude of the differences. Statistical significance was set at a p-value $<0.05$.

Furthermore, a generalized linear model (GLM) was used to establish the covariates of migratory behaviour in fishes. First, bivariate GLM model was fitted to establish the effect of independent variables (brain mass, soma volume, dendritic volume, dendritic length, dendritic complexity, dendritic volume, highest branch order and spine density) on migratory behaviour. Independent variables were considered as potential candidates when the p-value was less than 0.2, these variables could then be included in the final multivariable model used to predict covariates for migratory behaviour. Results were reported as coefficient and confidence intervals and p-value.

# CHAPTER 5: RESULTS

## 5.1. Results Summary

The cytoarchitecture of the telencephalon of the study species followed similar organisation with other teleosts (Giassi et al., 2012; Wulliman et al., 2014; Yamane et al., 1996). The dorsocentral pallium (Dc) had noticeably larger neurons, at lower density, than the neurons of the dorsolateral (Dl) and dorsomedial (Dm) pallium. Migrant species did not show any specific specialisation in Dl with regards to neuron morphology; in all species dorsolateral and dorsomedial pallium neuronal morphology were generally similar and differed significantly from those of the dorsocentral nucleus. Lastly, the generalised linear model did not find any neural correlate of migratory behaviour, however, spine density marginally predicted migrants.

## 5.2. General Cytoarchitecture of the Telencephalon

The forebrains of the species of interest followed the organisation previously described for other species (Giassi et al., 2012; Wulliman et al., 2014; Yamane et al., 1996). The telencephalon hemispheres were divided into dorsal (D) and ventral (V) areas. According to the theory of eversion in a teleost brain by Wullimann and Muellar (2004), the dorsal area of the forebrain is the everted area of the mammalian pallium, and the ventral area represents the subpallium. The dorsal area was made up of the following large cell masses; dorsodorsal (Dd), dorsolateral (Dl), dorsomedial (Dm), and dorsoposterior (Dp) surrounding a dorsocentral (Dc) region. The ventral region appears with divisions; superior (Vs), medial (Vm), and ventral (Vv).

In general, laterally, Dp and Dl were seen with a clear boundary, with Dl occupying a larger area than the region Dp. Dp which was generally seen to be posterolateral to Dl, was identifiable as an area of high density of cells which are darkly stained similar to observations in *Gymnotus sp*, as described by Giassi et al., (2012) (see Figure 5.2A). Dl is striated from the anterior to caudal band (as seen in Figure 5.1A, C and Figure 5.2A). More medially, Dc appears in the centre of the pallium characterised by the presence of the largest cells observed throughout the pallium (see Figure 5.1.A, D and Figure 5.2A, D), these cells are sometimes referred to as magnocellular (Mg) cells (Yamane et al., 1996) (see Figure 5.3A). Dc is bordered by Dp posteriorly and Dl laterally.

Most medially, Dc cells are observed more as well as covering a larger area in the centre of the pallium. Dm eventually replaces Dl and does not appear striated (see Figure 5.1B, E). Nissl-stained sections suggest that the neurons of Dm and Dl are of similar cell size (see Figure 5.1C, E and Figure 5.2C, E). The ventral region appears consistently from the lateral to most medial sections. However, the ventral region gets larger in volume until it covers most of the section followed by Dm (see Figure 5.1B and Figure 5.2B).

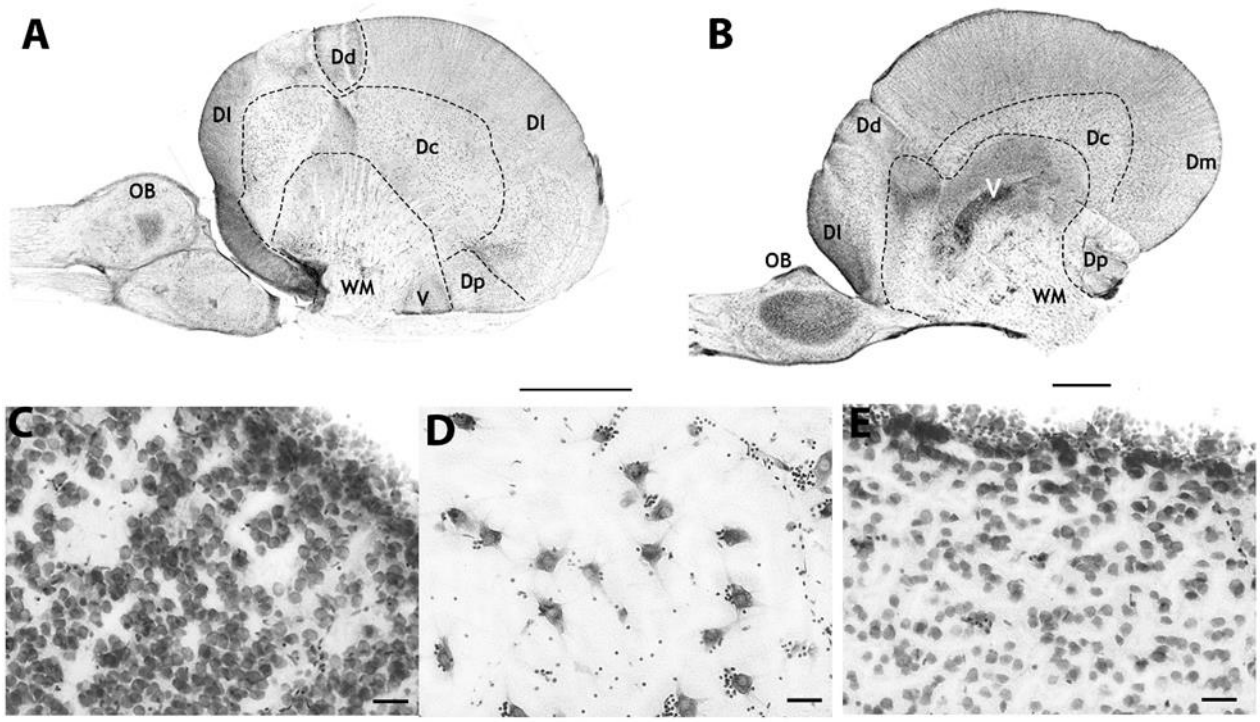



**Figure 5.1.** Photomicrographs of Nissl-stained sagittal sections of the telencephalon of *Diplodus capensis* (DC) brain. Whole brain virtual tissue scans (20x magnification) of the representative lateral **(A)** and medial **(B)** sections. The dorsocentral (Dc) region is evident in both sections by a lower cell density, than other regions. DC cells are considerably larger than other cells of the telencephalon, at high magnification (40x) and characterised by multipolar morphology **(D)**. More laterally **(A)**, the majority of the telencephalon is occupied by cells of the dorsolateral (Dl) region, which have a relatively higher cell density and are generally organised radially **(C)**. Cells of the dorsomedial (Dm) region are similar in size and morphology to cells of the dorsolateral (Dl) region, but do not show specific arrangement **(E)**. Cells of dorsodorsal (Dd) are seen as high density, small cells **(A, B)**. More medially **(B)**, a large part of the telencephalon is made up by ventral (V) regions. Also evident are cells of the dorsodorsal (Dd), ventral (V) and dorsoposterior (Dp) regions, clear boundaries are seen in the sections. OB-Olfactory bulb, WM-White matter. Scale bar A and B: 1mm. Scale bar C-E: 25μm.

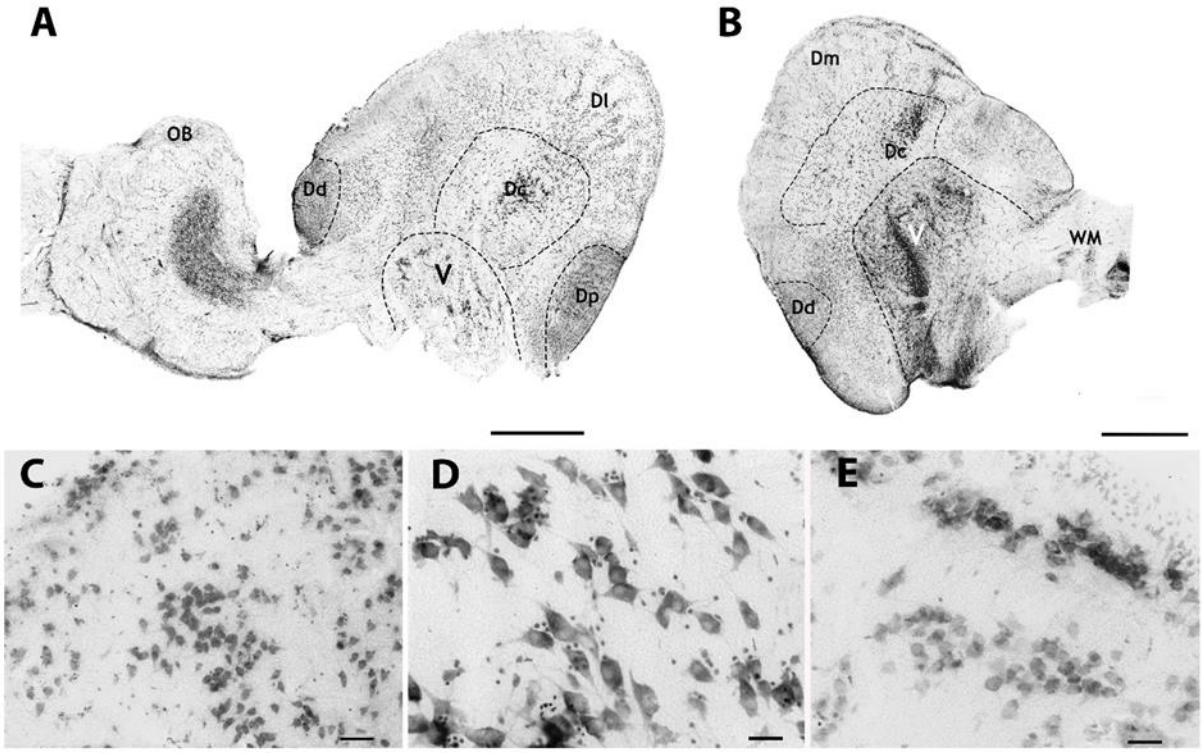



**Figure 5.2.** Photomicrographs of Nissl-stained sagittal sections of the telencephalon of *Pomatomus saltatrix* (PS) brain. Whole brain virtual tissue scans (20x magnification) of representative lateral **(A)** and medial **(B)** sections. Boundaries between different cell groups are less distinct than those of *Diplodus capensis* (fig. 5.1). The dorsocentral (Dc) region is evident in both sections by a lower cell density, than other regions. DC cells are considerably larger than other cells of telencephalon, at high magnification (40x) and characterised by multipolar morphology **(D)**. Cells of the dorsolateral (Dl, **C**) (20x) and dorsomedial (Dm, **E**) (40x) region have similar morphology **(C, E)**. Cells of dorsodorsal (Dd) are seen as high density, small cells **(A, B)**. More medially, the ventral (V) region makes up the majority of the telencephalon. OB-Olfactory bulb. WM-White matter. Scale bar A-B: 1mm. Scale bar C: 50 μm. Scale bar D and E: 25μm.

### 5.3 Microscopy of Golgi-stained Neurons

The peripheral regions of the sections were characterised by dense staining of radial glial cells. Radial cells appeared to be fan shaped with bipolar cell formation. The high density of radial cells is abruptly disrupted with a relatively less dense area with single neurons sparsely distributed in the Dl region. Dc is also an area of relatively high density in both migrants and residents. Similar to Mack et al., (2021),there were no radial glia observed in Dc region of migrants and resident brains. According to Mack et al., (2021) radial glia curves around the Dc region using radial glia so that new cells migrate to deeper pallial areas. Migrants notably consisted of patches of clustered cells followed by sparse cell regions unlike residents. Clustering in migrants was observed more in medial than lateral sections. Neuron types observed in both residents and migrants were multipolar, magnocellular multipolar, horizontal fork, inverted, unipolar and crab-like appearance according to descriptions by Yamane et al., (1996) and Jacobs et al., (2011). These cell types were randomly positioned across the section with no distinction of pallium except for magnocellular which occurred mostly in Dc. Examples of neuron types are displayed in Figure 5.3 and Figure 5.4

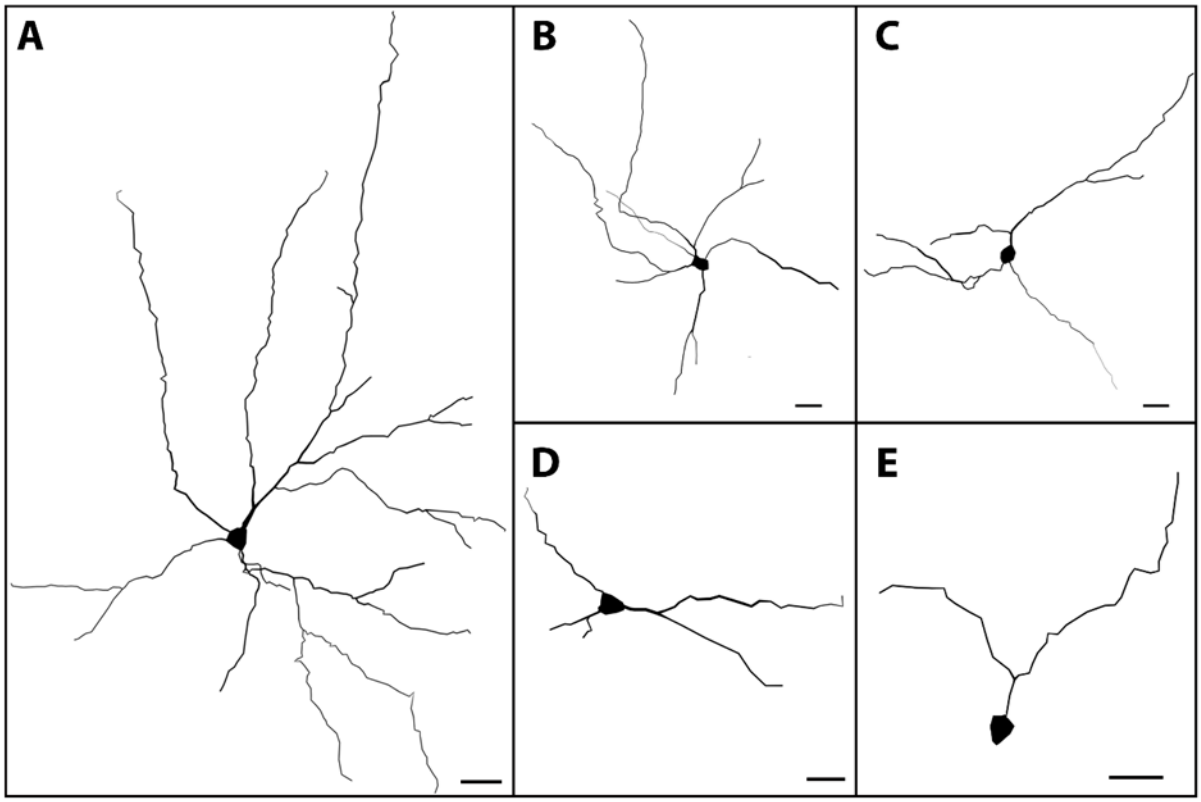



**Figure 5.3.** Two-dimensional Neurolucida tracings displaying cell types found in regions of the telencephalon: multipolar **(B, C)** dendrites proceed from more than three polar ends, magnocellular multipolar **(A)** described as a group with the largest multipolar cells, horizontal fork **(D)** described as having most dendrites arranged horizontally in opposite polar ends, bifurcated unipolar **(E)** dendrite processes only proceed from a single polar end. Neurons were traced from Golgi-stained neural tissue. Pial surface is on top of each drawing. Magnocellular multipolar neurons were only found in Dc pallium; while multipolar, horizontal fork, and bifurcated unipolar neurons were found across all divisions of the pallium. Scale bar in all tracings 25μm. Dc=Dorsocentral.

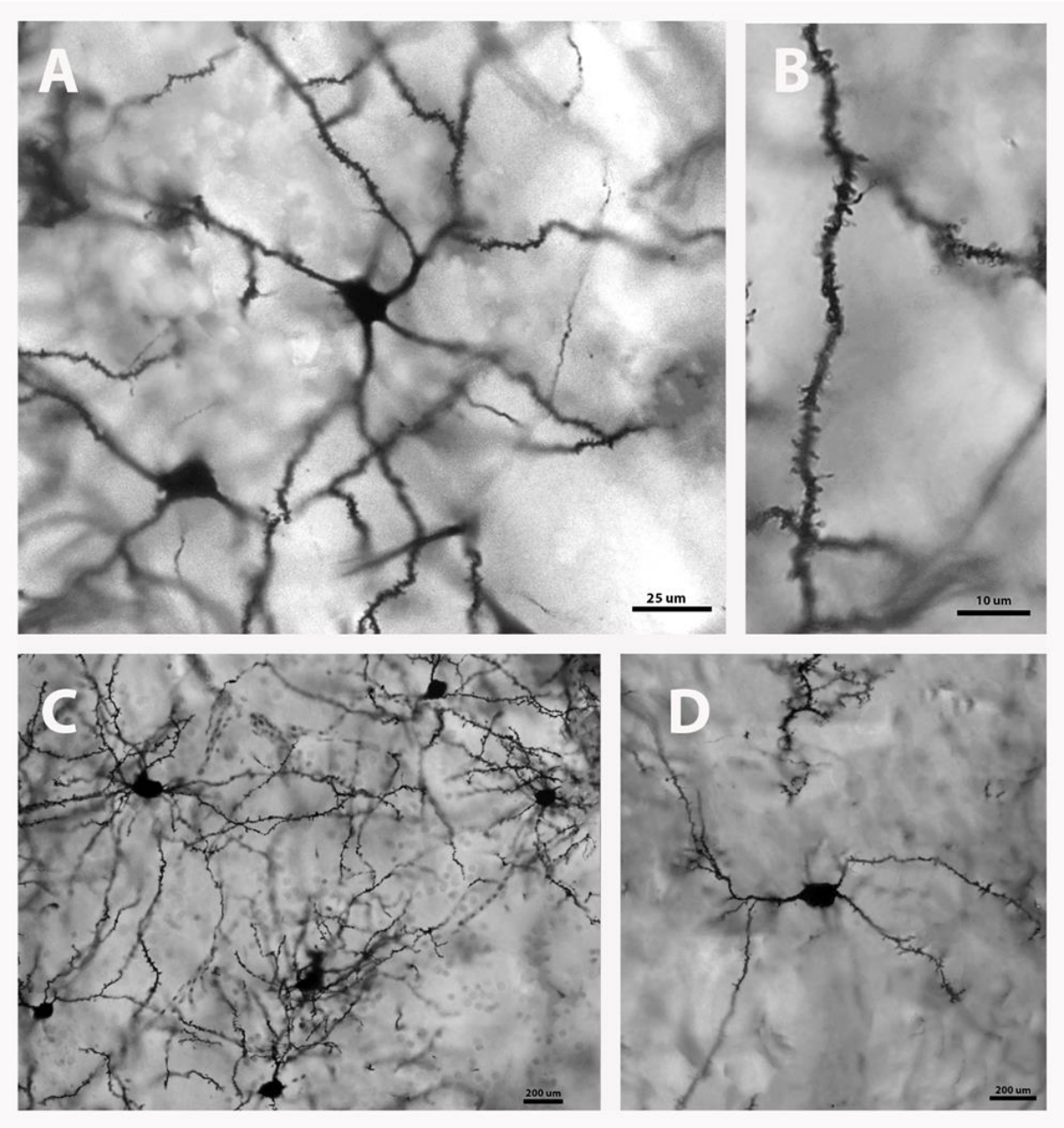


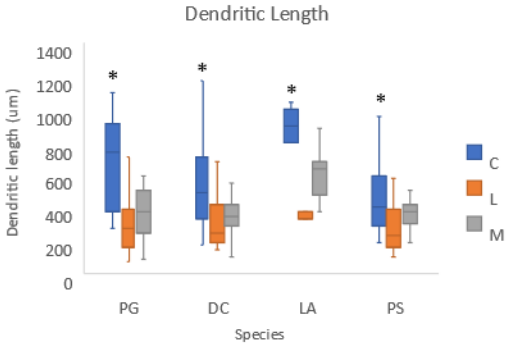

**Figure 5.4.** Photomicrographs of Golgi-impregnated neurons in the fish brain. **A**: Multipolar neuron from the dorsocentral (Dc) region of *Diplodus capensis* brain, 40x magnification, scale bar is 25µm. **B**: High magnification of dendritic spines of multipolar neuron (A), 100x magnification, scale bar 10µm. **C**: Neurons of the dorsomedial (Dm) region of *Pomatomus saltatrix* brain, 20x magnification, scale bar is 200µm. **D**: Horizontal neuron of the dorsolateral (Dl) region of *Pomatomus saltatrix* brain, 40x magnification, scale bar 200µm. All neural tissue were sagittally sectioned between 70µm and 100µm. Dendritic spines can be noted in all neuron types, there are clear differences in the dendritic volume; the volume of Dc neurons is greater than those of Dl and Dm neurons.

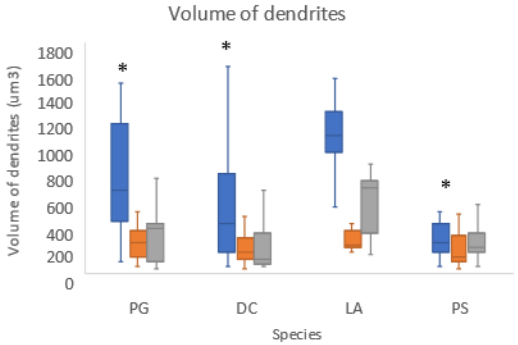
### 5.4. Morphometric Analysis of Neurons

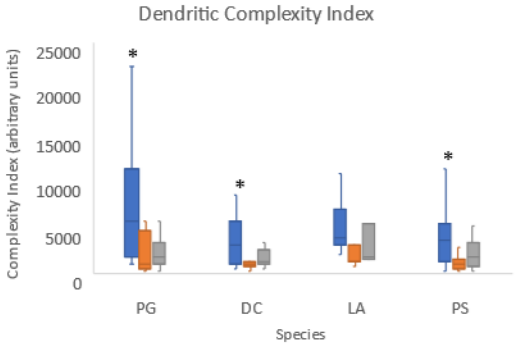
Differences in dendritic morphology in the dorsocentral, dorsolateral, and dorsomedial pallium were assessed and compared between resident and migrant species. In an effort to identify whether the neurons of the dorsolateral pallium showed any differences suggestive of specialisation of those neurons; various characteristics of neuronal morphology were compared between the regions of interest, for each species (as shown in fig. 5.5). The characteristics of neurons studied included dendritic length, dendritic volume, dendritic complexity, convex hull volume, spine density, highest branch order and soma volume.

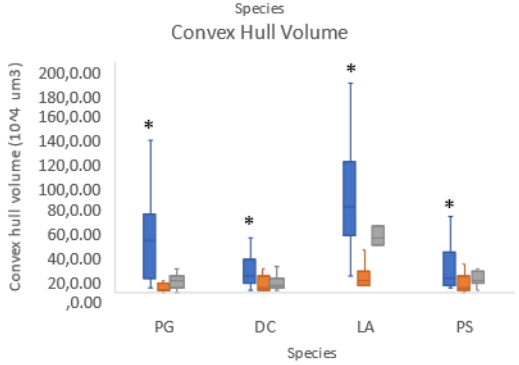
In general, no clearly discernible pattern was observed indicative of specialisation of Dl neurons. A significant difference in the total length of the dendrites of the neurons between the 3 regions of interest, with the neurons of the dorsocentral region showing significantly higher total dendritic length than those of the dorsomedial and dorsolateral regions. This was true in all species studied (fig. 5.5). Similarly, all species studied showed a significant difference in the convex hull volume of neurons in the different areas (fig. 5.5).

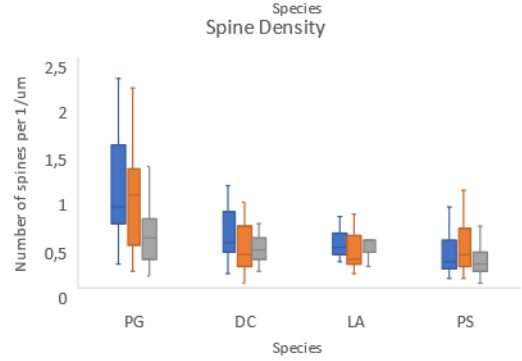
In contrast, the volume of dendrites, soma volume, dendritic complexity, and highest branch order, did not show significant differences in all species; they did not appear to follow a specific pattern (i.e., significant or non-significant only in migrants, or only in residents). The dendritic volume of the neurons in the 3 brain regions of interest varied significantly in both resident species studied (fig. 5.4, PG and DC) as well as one migrant species (fig. 5.5, PS); this was also observed in the case of the dendritic complexity index. The volume of the soma and highest branch order varied significantly only between the neurons of the species PG (*Pachymetopon grande*). While comparison of the density of spines per micron of the neurons in each brain region of interest showed no significant difference in any of the study species (fig. 5.5). In each case, the neurons of the dorsolateral and dorsomedial pallium were similar, and in cases where significant differences were observed, the neurons of the dorsocentral area varied the most. Table 5.1 gives a detailed analysis of the morphometric analysis of neurons.

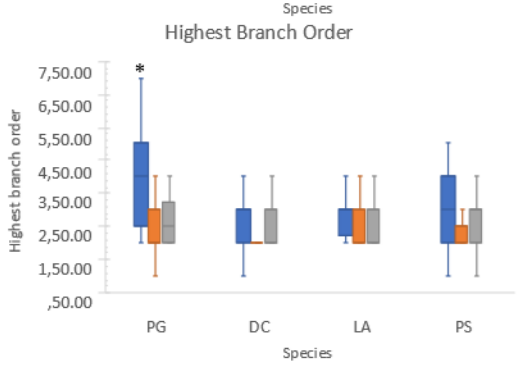

Dendritic Length
Dendritic length (um)
1400
1200
1000
800
600
400
200
0
PG
DC
LA
PS
Species
C
L
M


Volume of dendrites
Volume of dendrites (um3)
1800
1600
1400
1200
1000
800
600
400
200
0
PG
DC
LA
PS
Species


Dendritic Complexity Index
Complexity Index (arbitrary units)
25000
20000
15000
10000
5000
0
PG
DC
LA
PS
Species


Convex Hull Volume
Convex hull volume (10^4 um3)
200,0.00
180,0.00
160,0.00
140,0.00
120,0.00
100,0.00
80,0.00
60,0.00
40,0.00
20,0.00
,0.00
PG
DC
LA
PS
Species


Spine Density
Number of spines per 1/um
2,5
2
1,5
1
0,5
0
PG
DC
LA
PS
Species


Highest Branch Order
Highest branch order
7,50.00
6,50.00
5,50.00
4,50.00
3,50.00
2,50.00
1,50.00
,50.00
PG
DC
LA
PS
Species

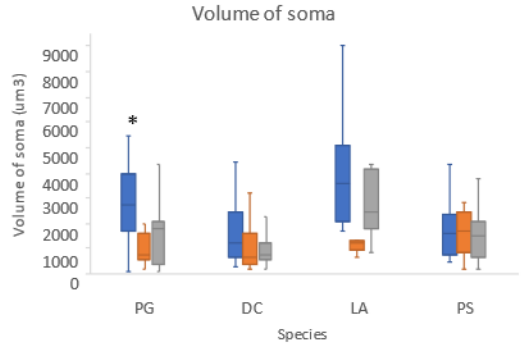

Volume of soma
Volume of soma (um3)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
PG
DC
LA
PS
Species

**Figure 5.5.** Box and whisker plots depicting the lack of specialisation of dendritic morphology in the dorsolateral (Dl) pallium compared to dorsocentral (Dc), and dorsomedial (Dm) pallium migrant species. **Residents: PG= *Pachymetopon grande* and DC= *Diplodus capensis*. Migrants: LA= *Lichia amia* and PS= *Pomatomus saltatrix***. Generally migrant species were similar to resident species, showing no specific patterns for example in volume of dendrites, volume of soma, dendritic complexity index, and highest branch order. A significant difference was found in dendritic length with neurons from Dc mostly different from neurons in Dl and Dm. Similarly, there was a significant difference in convex hull measurements, with Dc mostly different from neurons in Dl and Dm. The asterisk denotes significant relationships across Dl, Dc and Dm neurons which are represented as L, C, and M.

**Table 5.1.** Morphological analysis of neurons to assess specialisation in neurons of the dorsocentral (Dc), dorsomedial (Dm), and dorsolateral (Dl) region in residents (PG and DC) and migrant species (LA and PS). Interquartile range of different measured features on neurons was recorded along with p values. IQR =$Q_3$-$Q_1$ (Interquartile range= high quartile – lower quartile). C, L, and M corresponds to neurons of Dc, Dl, and Dm.

| **Fish specie** | ***Pachymetopon grande* (PG)** | | | | ***Diplodus capensis* (DC)** | | | | ***Lichia amia* (LA)** | | | | ***Pomatomus saltatrix* (PS)** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **IQR= Q3-Q1** | | | | **IQR=Q3-Q1** | | | | **IQR=Q3-Q1** | | | | **IQR=Q3-Q1** | | | |
| **Neuron feature** | **Dc** | **Dl** | **Dm** | **C vs M vs L p-value** | **Dc** | **Dl** | **Dm** | **C vs M vs L p value** | **Dc** | **Dl** | **Dm** | **C vs L vs M p-value** | **Dc** | **Dl** | **Dm** | **C vs M vs L p-value** |
| Volume of soma ($\mu m^3$) | 4478.18-1605.17 | 1615.34-514.55 | 2125.93-329.16 | 0.01 | 2530.06-690.85 | 1689.86-365.61 | 1351.56-510.71 | 0.06 | 6113.30-1887.81 | 2308.39-831.49 | 4255.59-1310.50 | 0.07 | 2406.32-729.31 | 2485.0-814.62 | 2247.04-640.77 | 0.82 |
| Volume of dendrites ($\mu m^3$) | 1287.51-403.07 | 348.99-102.26 | 404.42-57.49 | 0.001 | 828.16-162.83 | 283.88-106.04 | 360.87-79.78 | 0.006 | 1360.01-817.38 | 365.39-188.12 | 785.19-235.51 | 0.009 | 399.98-154.86 | 304.27-86.77 | 325.98-148.81 | 0.085 |
| Length of dendrites ($\mu m$) | 970.23-370.31 | 390.92-146.04 | 526.30-236.13 | 0.002 | 732.03-319.57 | 450.16-190.80 | 443.36-281.17 | 0.003 | 1014.45-693.45 | 505.78-269.89 | 773.97-429.10 | 0.01 | 645.92-291.90 | 391.86-158.67 | 424.86-293.89 | 0.026 |
| Highest branch order | 5-2 | 3.5-2 | 3.75-2 | 0.01 | 3.25-2 | 2-2 | 3-2 | 0.056 | 3.25-2 | 3.5-2 | 3.5-2 | 0.78 | 4-2 | 3-2 | 3-2 | 0.10 |
| Dendrite complexity index (DIC) | 13924.4-1572.51 | 4759.82-554.77 | 3588.76-859.02 | 0.007 | 5762.04-916.17 | 1295.12-710.20 | 2581.54-978.93 | 0.015 | 8476.56-2762.35 | 5645.19-1079.54 | 10133.1-1551.7 | 0.19 | 6009.56-1183.8 | 1521.88-601.91 | 1521.88-601.91 | 3420.88-795.96 |
| Convex Hull (Total volume) ($\mu m^3$) | 684910.3-124266.1 | 91860.07-13109.43 | 155636.7-22826.9 | <0.001 | 328429.6-81704.71 | 146869.9-21287.32 | 170283.2-43617.48 | 0.004 | 1324901-399535.8 | 285388.7-55911.45 | 800523.9-259135.6 | 0.027 | 427053.9-69971.15 | 151955.2-31366.6 | 186832.9-74037.5 | 0.034 |
| Spine density (1/$\mu m$) | 1.67-0.66 | 1.31-0.44 | 0.79-0.29 | 0.05 | 0.84-0.37 | 0.69-0.20 | 0.55-0.30 | 0.12 | 0.68-0.21 | 0.68-0.21 | 0.76-0.31 | 0.76 | 0.56-0.19 | 0.66-0.22 | 0.45-0.16 | 0.351 |

## 5.5. Volume Estimations of the Pallium

In residents, the proportional differences across these pallial areas reveal the dorsomedial pallium contributing the highest volume in the telencephalon (as seen in table 5.2). The neurons in this region had a convex hull neuron volume interquartile range between $1.4\times10^{-8}$ $cm^3$to $4\times10^{-9}cm^3$ and median volume of $6.6\times10^{-9}cm^3$. The dorsocentral pallium contributes the least volume to the telencephalon. The neurons in this region had a convex hull neuron volume interquartile range between $5.8\times10^{-8}cm^3$ and $1\times10^{-8}cm^3$, and a median volume of $1.8\times10^{-8}cm^3$. The dorsolateral pallium contributed a considerable volume. The neurons in this region had a convex hull neuron volume had an interquartile range of $9.3\times10^{-9}cm^3$ and $1.7\times10^{-9}cm^3$, and a median volume of $3.7\times10^{-10}cm^3$. The dendritic field of central neurons was greater than lateral and medial neurons as seen in Figure 5.6

In migrant individuals, the proportional differences across different pallial areas reveal the dorsolateral pallium contributing the highest volume to the telencephalon (as seen in table 5.2), the neurons in this region had a convex hull neuron volume with interquartile range between $1.6\times10^{-8}cm^3$ and $3.2\times10^{-9}cm^3$, and median volume of $5.8\times10^{-9}cm^3$. The dorsocentral pallium contributed the least volume to the telencephalon. The neurons in this region had a convex hull neuron volume interquartile range of $6.2\times10^{-8}cm^3$ and $7.2\times10^{-9}cm^3$, and a median volume of $1.5\times10^{-8}cm^3$. The dorsomedial pallium contributed a considerable volume to the telencephalon. The neurons in this region had a convex hull neuron volume interquartile range of $2.4\times10^{-8}cm^3$ and $8.1\times10^{-9}cm^3$ and a median volume of $1.2\times10^{-8}$ $cm^3$. The dendritic field of central pallium neurons was greater than lateral and medial neurons as seen in Figure 5.7.

**Table 5.2.** Analysis of pallial volume specialisation among migrant and resident species. Included is the dorsocentral, dorsomedial, dorsolateral to telencephalon volume ratio, and whole brain to telencephalon volume ratio. The CE represents the coefficient error of estimated volume measurement. A CE of 0.01 and less is considered normal and a close estimate of the volume of pallium.

| | **Volume estimation of telencephalon ($cm^3$)** | **Central pallium volume ($cm^3$) and [ratio to telencephalon]** | **Lateral pallium volume ($cm^3$) and [ratio to telencephalon]** | **Medial pallium volume ($cm^3$) and [ratio to telencephalon]** | **Average CE (Gunderson m=1)** | **Whole brain: telencephalon ratio** | **Average CE (Gunderson m=1)** |
|---|---|---|---|---|---|---|---|
| **Residents** | | | | | | | |
| Pomatopon grande (PG275) | 0.299 | 0.083[1:0.28] | 0.076 [1:0.25] | 0.0792 [1:0.26] | 0.07 | _ | _ |
| Diplodus capensis (DC258) | 0.243 | 0.049 [1:0.20] | 0.056 [1:0.23] | 0.090 [1:0.37] | 0.06 | _ | _ |
| Diplodus capensis (DC260624) | 0.320 | 0.073[1:0.23] | 0.062[1:0.19] | 0.053 [1:0.17] | 0.04 | 1:0.33 | 0.01 |
| Diplodus Capensis (DC270623) | 0.192 | 0.036[1:0.19] | 0.056[1:0.29] | 0.045 [1:0.24] | 0.04 | 1:0.30 | 0.00 |
| **Migrants** | | | | | | | |
| Pomatomus saltatrix (PS2706I) | 0.062 | 0.012[1:0.19] | 0.015[1:0.24] | 0.014 [1:0.23] | 0.05 | 1:0.12 | 0.01 |
| Pomatomus saltatrix (PS290631) | 0.05 | 0.007[1:0.13] | 0.014[1:0.28] | 0.0114 [1:0.23] | 0.03 | 1:0.11 | 0.01 |
| Lichia amia (LA280670) | 0.151 | 0.032[1:0.21] | 0.047[1:0.31] | 0.037 [1:0.24] | 0.02 | 1:0.12 | 0.01 |

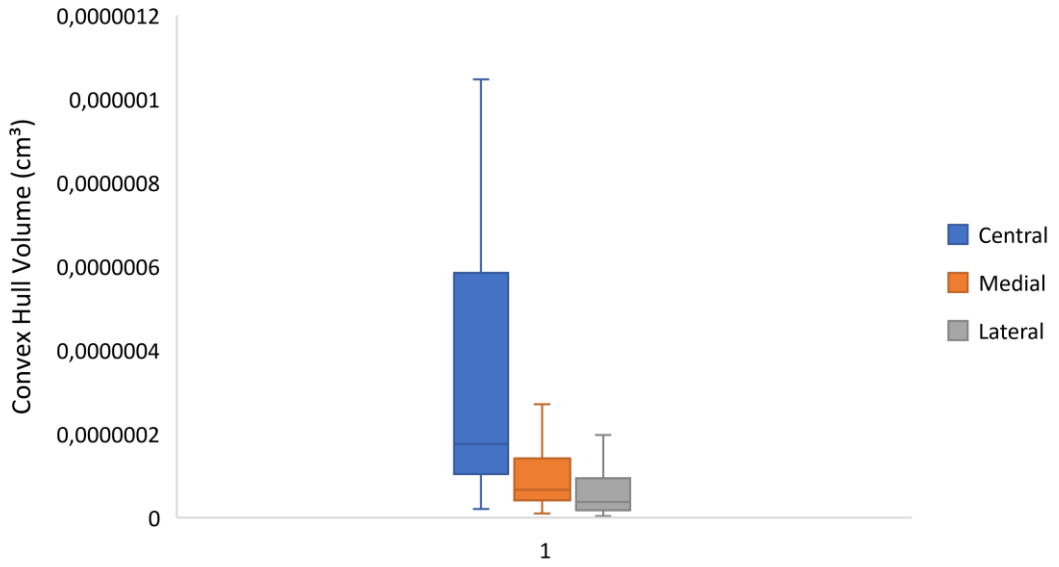



**Figure 5.6.** Convex Hull box plot depicting the highest dendritic field from dorsocentral neurons compared to dorsomedial and dorsolateral neurons in **residents**. Averages of convex hull measurements were obtained from reconstructed Golgi- stained neurons. Central neurons occupied the largest dendritic fields in resident species while lateral neurons occupied the least dendritic field. C, M, L correspond to Dc, Dm and Dl respectively. C [IQR]= $5.8\times10^{-8}$cm³ - $1\times10^{-8}$cm³, M[IQR]= $1.4\times10^{-8}$ $cm^3$- $4\times10^{-9}$cm³, L[IQR]= $9.3\times10^{-9}$cm³ - $1.7\times10^{-9}$cm³.

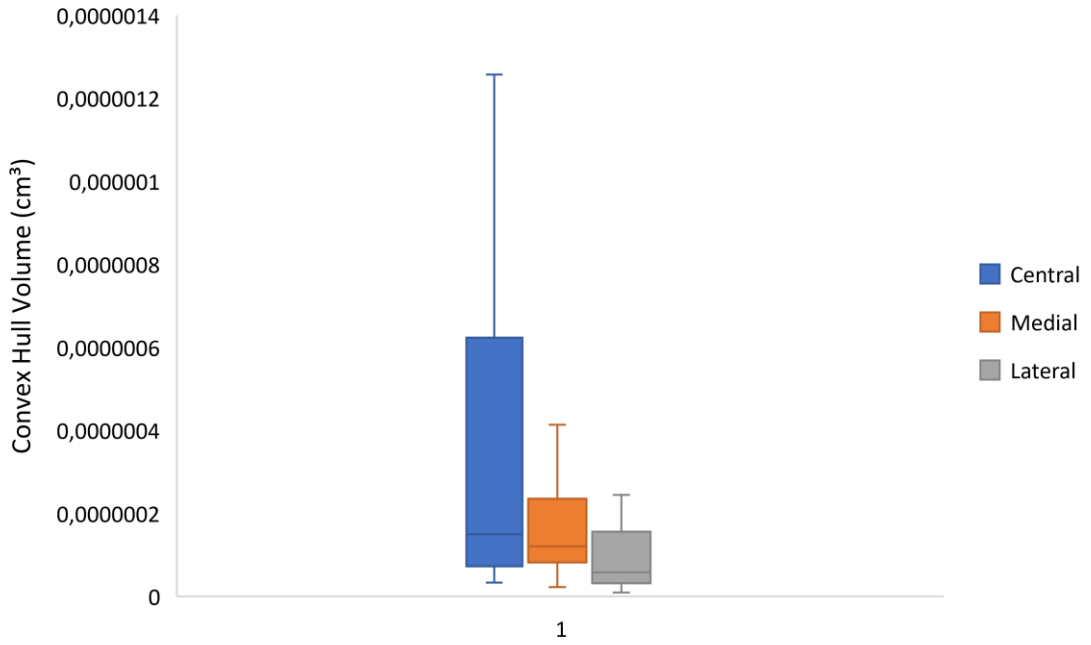



**Figure 5.7.** Convex Hull box plot depicting the highest dendritic field from dorsocentral neurons compared to dorsomedial and dorsolateral neurons in **migrants**. Averages of convex hull measurements were obtained from reconstructed Golgi- stained neurons. Central neurons occupied the largest dendritic fields in migrant species while lateral neurons occupied the least dendritic field. C, M, L correspond to Dc, Dm and Dl respectively. C [IQR]= $6.2\times10^{-8}cm^3$ - $7.2\times10^{-9}cm^3$, M[IQR]= $2.4\times10^{-8}cm^3$ - $8.1\times10^{-9}cm^3$, L[IQR]= $1.6\times10^{-8}cm^3$ - $3.2\times10^{-9}cm^3$. Central neurons had the largest dendritic fields in migrant species while the lateral neurons occupied the least dendritic field.

## 5.6 Generalised Linear Model

When the independent variable was compared against outcome variable of migration status in a bivariate model, highest branch order, volume of dendrites, and spine density were significantly different as seen in table 5.3. Dendritic volume was significantly lower by 1% in resident fishes compared to migratory fishes. Similarly, highest branching order was 11% lower in the migratory fish compared to resident after adjusting for other covariates. Spine density was reduced by 100% in migratory fish compared to resident fish.

A multivariable model set to predict migratory behaviour after being adjusted for brain mass, spine density, highest branch order, complexity of dendrites, and volume of dendrites. Spine density was marginally significant (95% CI: -1.59 – 0.13, p=0.096) see table 5.3.

**Table 5.3.** Bivariate and multivariable model depicting the lack of neural correlates of migratory behaviour.

| Correlates | | Bivariate model | | Multivariable model | |
|---|---|---|---|---|---|
| | **Total N** | **Coefficient (95% CI)** | **p-value** | **Coefficient (95% CI)** | **p-value** |
| Brain mass (g) **¥** | 176 | -1.50 (-3.65 - 0.65) | 0.173 | -1.04 (-3.26 – 1.17) | 0.36 |
| Soma volume ($\mu m^3$) | 214 | <0.01 (-0.00 – 0.00) | 0.614 | | |
| Dendritic length (μm) | 214 | -<0.01 (-0.00 – 0.00) | 0.479 | | |
| Dendritic volume ($\mu m^3$) **¥** | 214 | -<0.01 (-0.00 – -0.00) | 0.010* | 0.00 (-0.00 – 0.00) | 0.938 |
| Highest branch order **¥** | 214 | -0.11 (-0.16 – -0.07) | <0.001* | 0.10 (-0.07 – 0.28) | 0.239 |
| Dendritic complexity index **¥** | 214 | -<0.01 (-0.00 – 0.00) | 0.126 | -0.00 (-0.00 – 0.00) | 0.146 |
| Convex hull neuron volume ($\mu m^3$) | 214 | <0.01 (-0.00 – 0.00) | 0.327 | | |
| Spine density (1/μm) **¥** | 214 | -1.00 (-1.34 – 0.66) | <0.001* | -0.73 (-1.59 – 0.13) | 0.096 |

The coefficient depicts the direction and magnitude of the effect of the correlate in influencing migratory behaviours between migrant and resident fishes .

**¥** Denotes correlates selected and included in the multivariable model after fitting bivariate model because the p-values were <0.2. Correlates were ; ***** Denotes significant p-values<0.05; (95% CI) means 95% Confidence Interval

# CHAPTER 6: DISCUSSION

## 6.1. Summary of Findings

The current study aimed to investigate the neuronal morphology in the brains of *Pomatomus saltatrix* (PS) and *Lichia amia* (LA) (migrants) and *Pachymetopon grande* (PG) and *Diplodus capensis* (DC) (residents) to elucidate a relationship between neuronal morphology and migratory behaviour. We initially hypothesised that the brain regions and neurons shown to be involved in spatial navigation would show specific adaptations in migrants which are not seen in residents. This is based on studies in mammals and birds where species with advanced spatial navigation and memory abilities, show specialisation at the cellular level in the hippocampus (Sherry et al., 1992). However, the results show that overall, the telencephalon and neurons of the species studied are similar to those seen in previous studies and do show significant variation associated with the movement patterns in terms of telencephalon and dorsolateral pallium volume but, not in neuron morphology. Based on previous observation in other species, it is possible that spatial memory may not be the most important factor driving migratory behaviour. However, other sensory modalities and physiological factors do play a greater role in driving migration of the species.

## 6.2. The Cytoarchitecture of the Telencephalon

The telencephalon of all individuals studied followed the typical teleost formation consisting of prominent cell masses in the dorsal area of the telencephalon, namely the dorsodorsal (Dd), dorsolateral (Dl), dorsomedial (Dm), dorsocentral (Dc), and dorsoposterior (Dp) nuclei. Ventrally, superior (Vs), medial (Vm) and ventral (Vv) cell masses were observed. The morphology of the telencephalon of individuals of this study was similar to previous description of the telencephalon of other species, including, *Nothobranchius furzeri* (D'Angelo, 2013), and *Sebastiscus marmoratus* (Yamane et al., 1996), and mostly similar to *Danio rerio* (Wulliman et al., 2014) from which much of the nomenclature and cellular characteristics were based.

### 6.2.1 Radial glia allows for adaptation to growth processes

The presence of glial cells in the telencephalon is common for teleosts, with the radial glia being the most prominent type of astroglia (Mack et al., 2021), likewise glial cells were observed with fan shaped characteristic in brain tissue of all individuals. Bipolar radial cells

whose dendrites go towards the pial and ventricular surface were observed (Campbell & Götz, 2002) which is understood to be as a result of the eversion of teleost brains where cell bodies of radial glial cells are located at the brain surface surrounded by the ventricles (Mack et al., 2021). The presence of radial cells implies the ability of teleosts to grow and adapt to morphological growth processes (Mack et al., 2021). Particularly, the finding that radial cells curve around the Dc area is said to allow migration of new neurons to Dc areas allowing these neurons and glia to reach deep pallial areas. Dc was said to be the area of expansion in teleost like our observation of a relatively large Dc region in the telencephalon (Mack et al., 2021).

### 6.3. Specialization of the Lateral Pallium or Telencephalon in Migrants

Many of the specimens used in this study were donated by recreational anglers, thus it was not always possible to obtain body mass of the individuals studied in order to study the relationship between brain mass and body size. It was observed however, that the telencephalon contributed a relatively greater percentage of brain mass in residents (26.35%) than in migrants (8.76%). Furthermore, the dorsolateral nucleus (Dl) of PS contributed the largest volume (26%) to the telencephalon in contrast to the dorsomedial (23%) and dorsocentral pallium (16%), and in LA the dorsolateral nucleus contributed the largest volume (31%) in contrast to medial (24%) and central pallium (21%) (table 5.1). This marginal difference in volume may imply a specialisation in migrants that may facilitate spatial learning, spatial navigation and spatial memory (Vargas et al., 2009). Specialisation in spatial navigation has been correlated with a relatively larger hippocampal and hyperpallium volume as well as greater neuron density in migrating birds (Bernroider et al., 2014; Vincze, 2016), food caching birds and rodents (Sherry et al., 1992), and London taxi drivers (Maguire et al., 2000).

Bernroider et al., (2014) conducted a study comparing 7 species of short-distance songbird travellers and 8 species of long-distance songbird travellers in terms of their telencephalon and its subregions. Absolute and scalable brain volume measurements were taken, followed by a generalized encephalization quotient (EQ) calculated across the species. Similar to our findings, long-distance travellers had reduced relative and absolute brain volume, while their hippocampal volume was relatively larger than predicted (Bernroider et al., 2014). Similarly, Vincze (2016) compared breeding migrants and wintering (resident) birds of various phylogeny to elucidate brain morphometry with increasing migration distance. Across migratory behaviour, brain size was negatively correlated with migration distance greater than 1000km (Vincze, 2016), and the strength of this study is the 1466 bird species used over varying

geographical areas providing a more general association (Vincze, 2016). Both studies by Bernroider et al., (2014) and (Vincze, 2016) on migratory birds strongly suggest that the observation seen in our study, follow typical vertebrate patterns.

Bernroider et al., (2014) proposes that resident birds are evolutionarily selected with larger telencephalon volume because of their developmental mechanisms compared to behavioural adaptations. Precursor telencephalon cells of residents remains longer in the proliferative cell cycle resulting in enlargement of their telencephalon (Bernroider et al., 2014). It would be of interest to study whether the same is seen in developing teleosts.

It is also possible that the observed differences in the relative brain size among migrants and residents is related to the energetic trade-off and behavioural flexibility hypothesis (Winkler et al., 2004). Migrant fish are energetically limited during migration season due to the food deprivation or reduced feeding associated with migration (Binder et al., 2011) leading to all cognitive and energy resources focused on reaching the destination. The findings of Vincze (2016) provide support for energetic trade-off and behavioural flexibility in migrant birds and higher cognitive need in residents hence their relatively larger brain size. Reduced brain size (of migrants) was strongly correlated with milder wintering temperatures at the migrants destination, meaning that migrants who travelled far distances preferred milder winter temperatures where finding food would not be energetically expensive (Vincze, 2016). Therefore, this meant that resident birds that stayed in one habitat left by migrants would weather the harsh conditions to feed and survive, thus the higher cognitive need (Vincze, 2016). Certainly, migrants did not have decreased cognitive need during migration rather reduced brain size occurred as a result of the energy trade-off in birds (Vincze, 2016).

The result presented in the current study would be strengthened by an increased sample size, however, the result of studies like Bernroider et al., (2014) with sample sizes of 83 birds across 15 species, and Vincze (2016) with sample size of 1466 bird species that are phylogenetically related show that there is a general trend of a relatively larger brain size in resident species compared to migrants. Additionally, in both mammals and bird, studies have shown relatively larger hippocampal volume to be associated with specialisation in spatial navigation skills (Sherry et al., 1992), adding support to the results presented in this study. Admittedly, an increased sample size would strengthen the CE to 1% or less, studies such as (Dell et al., 2015) corroborate this suggestion.

Notably, in migrants the marginal increase in dorsolateral pallium volume did not result in the increased dendritic field of the neurons. Dorsocentral pallium neurons dendritic field was highest even though the volume of the pallium was the least. This finding implies that increased volume of brain region does not directly reflect the size of neurons nor their connectivity. My finding corroborates a study by Herculano-Houzel (2015), indicating a trade-off occurs where increased area in the brain may be accompanied by increased cell densities but reduced dendritic fields and soma size. Further stereological estimates of neuronal number in the telencephalon would be beneficial in understanding this relationship.

### 6.4. The Lack of Specialisation in Lateral Pallium in Neuron Morphology

Although the telencephalon of migrant did not contribute the largest volume to brain mass, their dorsolateral pallium had a greater volume compared to residents. The analysis of dendritic morphological changes in the current study did not show any specific specialisation in the neurons of the dorsolateral pallium in relation to their function in spatial navigation. In both residents and migrants, Dl neurons were similar in volume of soma, convex hull, dendritic length, dendritic volume, dendritic complexity index, spine density, and highest branch order to those of Dm; whereas marked morphological differences were observed between the neurons of the Dc pallium and those of the Dm and Dl neuron groups. In general, since Dc to Dl and Dc to Dm groups were similar in migrant and resident species, dorsocentral neurons were the common difference.

The dorsocentral pallium (Dc) has been regarded as an area of integration and modulation of subpallial areas (for example Dm and Dl) (Demski, 2013), cognitive and emotive processing (Mueller et al., 2011). Dc's interconnections to Dm and Dl reportedly facilitates its processing of various sensory modalities from the diencephalic nuclei and sending efferent projections to the cerebellum (Demski, 2013). Making Dc a major processor of sensory modalities such as vision, auditory, olfactory and output modulator in the teleost brain (Demski, 2013). Dc neurons should appear different in morphology compared to dorsolateral and dorsomedial pallial neurons due to dorsocentral pallium interconnections and its high-level processing. These morphological differences were observed in dendritic volume, convex hull, dendritic complexity index, and volume of dendrites in migrants and residents (Figure 5.3).

Several studies have provided evidence of hippocampal changes associated with avian migratory behaviour. For example, migratory subspecies of dark eyed Junco (*Junco hymalis*) have a greater density of hippocampal neurons than the resident subspecies, although

hippocampal volume between them was not significantly different (Cristol et al., 2002). The migratory Gambel's white-crowned sparrow had a relatively larger hippocampus than the resident Nuttall's white-crowned sparrow (Pravosudov et al., 2006). The study also found age to be a factor in hippocampal neuroplasticity, where adult Gamble's white crowned sparrows had a relatively larger hippocampus than adult Nuttall's white-crowned sparrows (Pravosudov et al., 2006). Additionally, LaDage et al., (2011) observed increased hippocampal neurogenesis in migratory Gamble's white-crowned sparrow than resident Nuttall's white-crowned sparrow.

In these studies, a clear relationship was identified between the hippocampus and migratory behaviour. In contrast soma volume, dendritic length, dendritic volume, complexity of dendrites and highest branch point were not significantly related to migratory behaviour. This potentially relates to the reduced sample size in the current study, as well as the phylogenetic unrelatedness between study species. However, it is also possible that my findings are reflective of the impact of seasonal changes  or associated migratory stress; as increased cognitive demand, exercise and stress factors are viewed to influence seasonal changes in the avian hippocampus (Sherry & Macdougall-shackleton, 2014).

Nevertheless, it is likely my findings suggest that migrants could be relying on other sensory cues to get to their destination more than relying on spatial memory of geographical location of their destination. Long distance migrants are thought to be using geomagnetic and olfactory maps for position fixing in open water giving migrants a crude direction and orientation for them to stay on course to their seasonal spawning home (Luschi, 2013). Such maps have been conceptualized from sea turtle and seabird studies which seem to rely on geomagnetic and olfaction cues (Luschi, 2013). Furthermore, Putman et al., (2014) describes Chinook salmon responding to magnetic fields when orienting themselves in relation to their homing habitats. Other biological cues that assist fish with maintaining direction in their environment include the sun (Luschi, 2013) or polarized light when the sun is obscured (Binder et al., 2011). Relying more on sensory cues seems to have less remodelling capacity for neuron morphology in the dorsolateral pallium which aligns with my findings. The reduced reliance on spatial memory and consolidation of allocentric and egocentric cues may be the reason for my observations.

The differences between dorsolateral and dorsomedial neurons between closely related species should reflect a difference in the physical and social environment (Shumway, 2008), and migratory behaviour as seen in mammals, birds and rodents specialized in spatial navigation (Sherry et al., 1992). In the current study, both residents and migrants had similar physical

environments namely the ocean, and social environment namely, schooling activity and exposure to predators. The similarity of the morphology of these neurons may be because of these similarities in environment and social environment.

### 6.5. The Neural Correlates of Migratory Behaviour

The current study found a similar amount of spine density in residents and migrants, this implies that in species studied, spine density does not differ regardless of migratory behaviour. However, Brinkman, (2019) found that spine density plays a role in behavioural changes in Richardson's ground squirrels (*Urocitellus richardsonii*), breeding season is marked by a significant loss of spine density, and conversely, recovery during hibernation period. But the current study found otherwise, the differences in the findings could be influenced by the small sample size of the current study because the results showed a marginal correlation between spine density and migratory status ($p=0.096$). This result could be attributed to the disparate effects of the various stimuli have on spine density during seasonal migration, namely physiological stress and cognitive demand associated with a changing induced environment (Bernroider et al., 2014). Thus, the need to measure glucocorticoid levels during fish seasonal migration in future studies.

Similar to a study by Sherry et al., (1992), our findings found a relatively larger lateral pallium in migrants with extensive spatial skills. Looking at neuronal level in the hippocampus, the larger hippocampus did not translate to increased soma volume, volume of dendrites, and convex hull (i.e., the volume occupied by the individual neuron). Similarly, Herculano-Houzel, (2015) found that the finding of a relatively larger area in the brain may increase neuron density but not necessarily the size of neurons and dendritic fields.

Despite the relatively larger telencephalon of residents contributing more to its brain mass than the brain mass of migrants, reducing brain mass was not significantly correlated to migrants in the generalised linear model. According to literature, the relative brain mass of migrating birds is expected to reduce (Bernroider et al., 2014; Vincze, 2016). Conversely, my findings imply that brain mass does not take part in migratory behaviour in the studied species. Given the evidence in avian migration an increased sample size would reflect a similar trend to avian migrants, where they would have reduced brain mass (Vincze, 2016).

Changes in spine density, soma volume, brain mass, dendritic length and convex hull can occur because of seasonal changes to adapt to changes in that environment, or behaviour such as

migration and breeding, and stress. Many studies are trying to isolate the effect of each variable to changes in hippocampal neuroplasticity using a combination of before and after migration studies in closely related species, using wild caught animals in their natural environments in comparison to lab-based experiments.

The current study shows that in resident and migratory species which are not closely related, measurements of neuron morphology could not be clearly associated with any migratory behaviour. Additionally, specialization of the dorsolateral pallium in migrants was at the level of relative hippocampal volume, relative reduced telencephalon volume, and reduced brain mass but not neuronal morphology.

### 6.6. Future Studies

Due to a general lack of neuroplasticity measurements in terms of neuron morphology. Genetic markers for neuroplasticity and neurogenesis markers in the different division of the pallium

could help establish a finer distinction of changes occurring in Dl, Dm and Dc in migrant and non-migrant species. Furthermore, examining other pallial areas like Dd and Dp could help our understanding of spatial navigation strategies utilized by teleost fish.

As with findings implying that fish may be using sensory cues more than spatial memory in spatial navigation, looking at areas that process sensory cues such as the optic tectum would be valuable to find neuronal correlates of migratory behaviour.

With our findings showing that effects of chronic stress are not always reflected in neuronal morphology changes, an addition of behavioural and physiological tests would help assess chronic stress differences between residents and migrants. Future studies should investigate whether loss of dendritic spines is in relation to changing season or chronic stress since species we used migrate for spawning reasons. Body measurements to reveal muscle atrophy before and after migration season could be assessed. In our data collection we failed to collect blood from both resident and migrant groups in order to establish their cortisol levels which could have provided stronger association of stress and pallium volume changes.

### 6.7. Conclusion

The current study suggest that migrants display an adaptation to migratory behaviour by having a smaller absolute brain mass, of which the telencephalon makes up a relatively smaller proportion. Like other vertebrates that rely on spatial navigation, the hippocampal/dorsolateral

pallium became adapted by displaying a relatively larger volume of the telencephalon than the dorsomedial and dorsocentral pallium. Contrary to our expectation, the neurons of the dorsolateral pallium showed no specialisations of their morphology; the generalised linear model did not show neural correlates of migratory behaviour suggesting that migrants in this study may have been relying less on map-like spatial memory formation for navigation rather other sensory cues.

Given the similarities observed between the current study, and studies in birds, rodents, and mammals as it relates to hippocampal adaptation, fish brains could be used as a model to study brain adaptation of different behaviours facilitated by the hippocampal pallium. Also, fish can reliably be used to understand what it takes to migrate since they are not that different to other animals. Their increased neuroplasticity in adult hippocampus and even during injury makes them a good candidate to study the effects of anxiety, mood, depressive disorders and neurodegenerative diseases which have a strong correlation to chronic stress.

Additionally, there remains a need for further protection of migrating species in their migratory routes through increased surveillance of fishing sexually immature fish. To fight reducing fish populations impacted by climate change which further affect fish migration apart from internal demands of migration in fish.

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## APPENDICES

### 1. ETHICS

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

**STRICTLY CONFIDENTIAL**

**ANIMAL RESEARCH ETHICS COMMITTEE (AREC)**

**CLEARANCE CERTIFICATE NO.** 2019/05/34/C

**APPLICANT:** Dr A Ngwenya

**SCHOOL:** Anatomical Sciences
**DEPARTMENT:**
**LOCATION:**

**PROJECT TITLE:** Comparisons of cytoarchitecture and neuronal morphology in the brains of migratory (Leervis; Lichia amia and elf/shad; Pomatomus saltatrix) and resident (Blacktail; Diplodus capensis and red roman; Chrysoblephus laticeps) fish species

**Number and Species**

15 max adult Blacktail (Diplodus capensis), 15 max adult Red roman (Chrysobephus laticeps), 30 max adult Leervis (Lichia amia) and 30 max adult Elf/shad (Pomatomus saltatrix)

Approval was given for the use of animals for the project described above at an AREC meeting held on 2019/05/28. This approval remains valid until 2021/07/07.
Unreported changes to the application may invalidate the clearance given by the AREC
An annual progress report must be provided

The use of these animals is subject to AREC guidelines for the use and care of animals, is limited to the procedures described in the application form and is subject to any additional conditions listed below:

Signed: _____ (Chairperson, AREC) Date: 15th July 2019

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ (Registered Veterinarian) Date: 11 July 2019

cc: Supervisor: N/A
Director: CAS

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**Please note that only typewritten applications will be accepted.**

# UNIVERSITY OF THE WITWATERSRAND

## ANIMAL ETHICS SCREENING COMMITTEE
## MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. **Name**: Ayanda Ngwenya

b. **Department**: School of Anatomical Sciences

c. Experiment to be modified / extended

| | | | AESC NO |
|---|---|---|---|
| Original AESC number | **AESC19** | **05** | **003** |
| Other M&Es : | | | 1 (16 April 2020) |

d. **Project Title**: Comparisons of cytoarchitecture and neuronal morphology in the brains of migratory (Leervis; *Lichia amia* and elf/shad; *Pomatomus saltatrix)* and resident (Blacktail; *Diplodus capensis* and red roman; *Chrysoblephus laticeps*) fish species

| | **No.** | **Species** |
|---|---|---|
| e. Number and species of animals originally approved: | • Up to 15 of each resident species.<br>• Up to 30 of each migrant species | • Resident species:<br>  ○ Blacktail (*Diplodus capensis*)<br>  ○ Red roman (*Chrysoblephus laticeps*)<br>• Migrant fish species:<br>  ○ Garrick/Leervis (*Lichia amia*)<br>  ○ Elf/shad (*Pomatomus saltatrix*) |
| f. Number of additional animals previously allocated on M&Es: | 15 | Resident species: Bronze bream (*Pachymetopon grande*) |
| g. Total number of animals allocated to the experiment to date: | 75 | |
| h. Number of animals used to date: | 13<br>12<br>1<br>15 | Elf/shad (*Pomatomus saltatrix*)<br>Blacktail (*Diplodus capensis*)<br>Garrick (*Lichia amia*)<br>Bronze bream (*Pachymetopon grande*) |

**i. Specific modification / extension requested:**

Extension of study for study until December 2023.
Addition of 2 more migrant species:
Dusky kob (*Argyrosomus japonicus*), up to 15 individuals.
Southern African pilchard (*Sardinops sagax*), up to 30 individuals.

**j. Motivation for modification / extension:**

Extension of study:
Due to restrictions placed on travel and recreational fishing as a result of the covid 19 pandemic and lockdown, it was not possible to travel for specimen collection for much of 2020. As the study requires

specimens at different points of the migratory journey, we require extension of the study.

Additional migrant species:

Garrick (*Lichia amia*) are a popular sport fish as they can be quite challenging to catch. Additionally, the bag limit is low (each angler may only keep 1 individual per day), and the fish caught must measure at least 70 cm in order for it to be kept. We have been able to collect sufficient specimens of shad (the other migrant species), but it has not been so easy to collect Garrick. In order to reliably investigate the relationship between neuronal morphology and movement patterns, it is necessary to include additional migrant species, specifically dusky kob (*Argyrosomus japonicus)* and sardines (*Sardinops sagax*). Kob are another popular angling species which have been shown to undertake spawning migration, but like Garrick, there are strict limits on the number and minimum size of individuals (Griffiths, 1996). Of particular interest is the annual sardine run on the KwaZulu Natal coast, which draws many recreational anglers as the mass movement also acts as a draw for many marine predators (including Garrick, shad and kob), thus it will be beneficial to collect several different species of interest in sampling trips. The drivers of the sardine migration are not yet well understood, with some authors suggesting that the movement is largely driven by currents (and not related to feeding or spawning), and others reporting that most specimens caught during the migration were reproductively active (Van der Lingen et al. (2010). Thus, the addition of this species has the potential to elucidate whether this species shows specific neural specialisations, associated with long distance migration. This species is easily and regularly caught by recreational anglers (and even novice fishers) during the sardine run and has not been listed as threatened by the IUCN.

References:


- Van Der Lingen, C.D., Coetzee, J.C. and Hutchings, L., 2010. Overview of the KwaZuluNatal sardine run. African Journal of Marine Science, 32(2), pp.271-277.

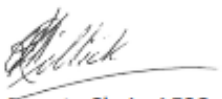
- Griffiths, M. H. (1996). Life history of the dusky kob Argyrosomus japonicus (Sciaenidae) off the east coast of South Africa. South African Journal of Marine Science, 17(1), 135-154.


**Date:** 17/08/2021 **Signature:**

**RECOMMENDATIONS:**

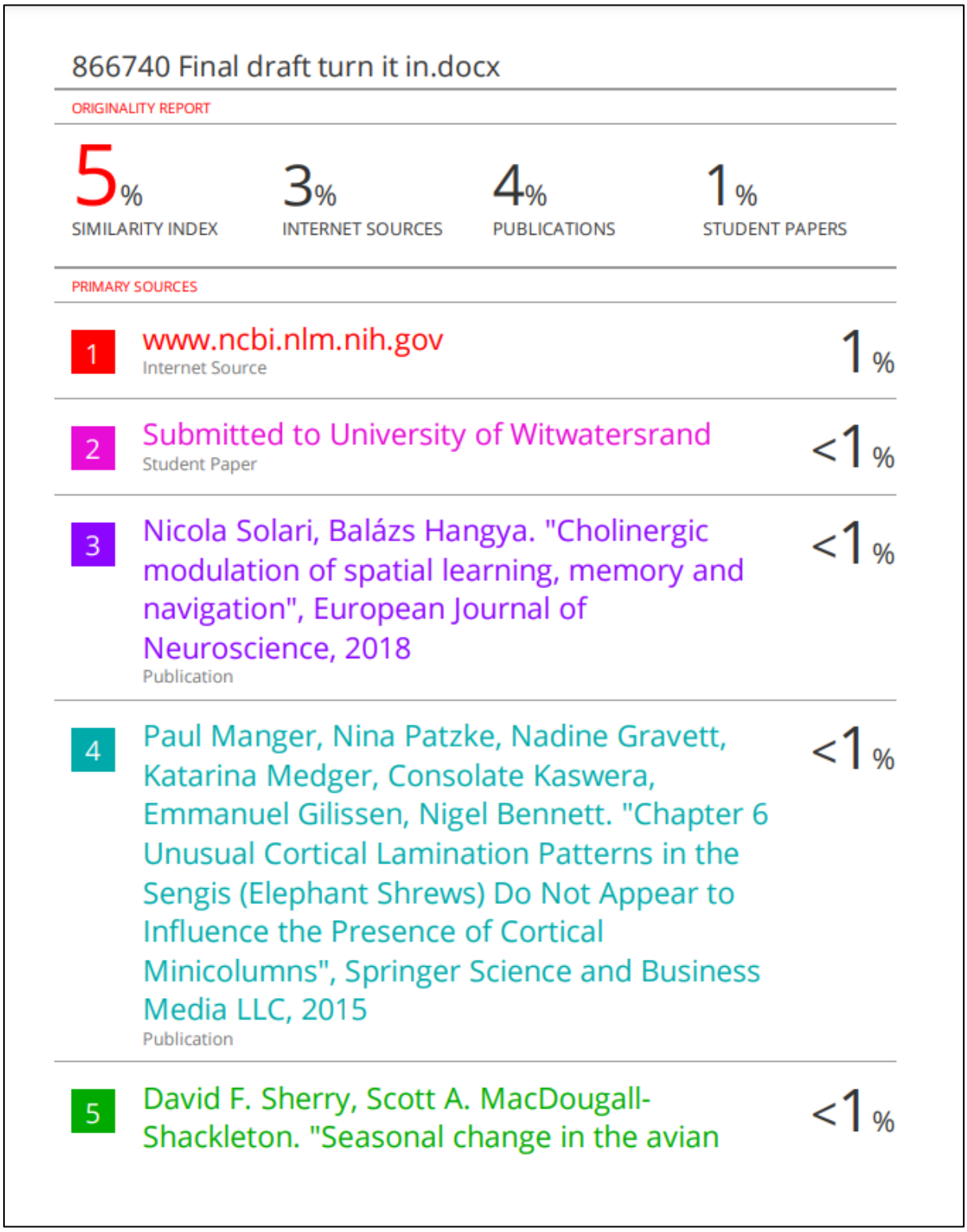
Study extension granted until 31 December 2023. Please ensure that any additional extension of time is applied for prior to the new expiry date (i.e. 31 December 2023).
Approval for the addition of two migratory species to the study (*Argyrosomus japonicus* and *Sardinops sagax*) as detailed above. The researcher is to ensure that the termination of fish species is carried out in accordance with original approved study methodology (200-300 mg clove oil per litre of water) and that the newly requested species are included on the respective provincial/national permits.

Date: 19 August 2021 Signature:



Deputy Chair, AESC

## 2. SIMILARITY REPORT



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