UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2022/23
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Name of student:

Daiki Sugita

Twitter Handle*:
*(*optional)*

Name of supervisor(s):

Professor Clare V. H. Baker

Project Title: (no more than 220 characters)

Studying the development of independently evolved electroreceptors in teleost fishes

Project aims: (no more than 700 words)

Background:
Mechanosensory hair cells in the inner ear, arising from the otic placode, mediate hearing and balance. In fishes and amphibians, almost identical hair cells are also found in lateral line placode-derived neuromasts, distributed in lines over the head and trunk, which detect local water flow. In electroreceptive species (e.g. skates, sturgeons, salamanders), some or all of the cranial neuromast lines are flanked by fields of electrosensory 'ampullary organs' containing electroreceptor cells, which detect weak, low-frequency, electric fields around other animals in water (primarily used for hunting live prey). Lateral line placodes form ampullary organs as well as neuromasts, and that electroreceptors are closely related to hair cells (reviewed in Baker et al., 2013; Baker & Modrell, 2018). Electroreception was lost in the lineages leading to teleost fishes and to frogs, so electroreceptor development cannot be studied in the standard lab models, i.e., the teleost zebrafish and the frog *Xenopus*.

However, electroreception evolved at least twice independently within teleost fishes, most likely from neuromast hair cells (Baker et al., 2013) - a superb example of convergent evolution. Low-frequency 'ampullary' electroreceptors, which are physiologically different from non-teleost electroreceptors but also innervated by lateral line nerves, evolved independently in ostariophysan catfishes and their sister group, the knifefishes, and in osteoglossomorph mormyroids and their sister group, the notopterids. Knifefishes (which include the electric eel) and mormyroids also independently evolved electric organs (hence are 'electric fish') and high-frequency 'tuberous' electroreceptors that respond to distortions in their self-generated electric field, enabling e.g., electrolocation in muddy waters and communication. Tuberous electroreceptors could have evolved either as a specialisation of ampullary electroreceptors, or via a second independent modification of neuromast hair cells.

Histological descriptions of neuromast and electroreceptor organ development are available for two ostariophysan teleosts: the channel catfish, *Ictalurus punctatus* (Northcutt et al., 2000; Northcutt, 2003) and the knifefish *Eigenmannia* (Vischer, 1989a,b; Vischer 1995). Catfish ampullary organs and knifefish ampullary and tuberous organs develop next to neuromast lines, consistent with a lateral line placode origin (though this has not been tested experimentally). No molecular information has been published on teleost electroreceptors, although preliminary *in situ* hybridisation and immunostaining data for channel catfish embryos were available in the lab. Furthermore, our collaborator Harold Zakon (UT Austin, Texas, USA) has an unpublished dataset of candidate genes potentially expressed in electroreceptor organs of a knifefish (in a sister group to catfishes). This dataset arose from differential expression analysis of transcriptome data from electroreceptor organ-rich skin versus electroreceptor organ-poor skin dissected from an adult black-ghost knifefish (*Apteronotus albifrons*).

Aims and Objectives: We aimed to test the hypotheses: (1) that teleost electroreceptor organs evolved as modified neuromasts, by comparing expression of known neuromast hair cell and support cell markers; (2) that electroreceptors evolved in the common ancestor of catfish and knifefish, by analysing the expression of a variety of candidate genes from the knifefish electroreceptor organ-enriched dataset.

Project Outcomes and Experience Gained by the Student (no more than 700 words)

Approach:
In situ hybridisation (in wholemount and on sections) and wholemount immunostaining were used to study the expression of a range of candidate genes - mostly encoding transcription factors - in embryos and larvae of the channel catfish (*Ictalurus punctatus*). Shared transcription factor expression in neuromasts and ampullary organs may support shared developmental mechanisms, and potentially a shared evolutionary relationship (consistent with the evolution of ampullary organs as modified neuromasts). Some candidate genes were selected from a putatively electroreceptor organ-enriched gene-set, arising from differential RNA-seq analysis of skin transcriptome data from the black ghost knifefish (*Apteronotus albifrons*), in a sister group to catfishes. Expression in catfish ampullary organs of candidate genes from the knifefish electroreceptor organ-enriched gene-set would be expected if ampullary organs evolved in the common ancestor of catfishes and knifefishes.

cDNA fragments for catfish homologues of candidate genes selected from the knifefish electroreceptor organ-enriched skin dataset (encoding e.g., transcription factors, signalling pathway receptors) were either already available in the lab as templates for riboprobe synthesis, or identified from catfish transcriptome data and synthesised as part of the project.

Catfish embryos were staged according to Armstrong (1962). Neuromasts and ampullary organs were identified based on their distribution in developing, juvenile and adult channel catfish (Northcutt et al., 2000; Northcutt, 2003). Many experiments used post-feeding larvae, when the lateral line organs are functional. Embryonic stages were also investigated for some genes: stage (S)35, when lateral line placode-derived sensory ridges are present but before neuromast primordia are detectable histologically (Northcutt, 2003); S43 (hatching), when neuromast primordia have formed and the first ampullary organ primordia appear, and a later yolk-sac larval stage, when more ampullary organ primordia have formed (Northcutt, 2003).

Outcomes:
The expression of 19 genes - mostly encoding transcription factors - was examined. A handful of candidates from the knifefish dataset proved not to be expressed in lateral line organs. However, most genes tested were expressed in both neuromasts and ampullary organs, including transcription factor genes expressed in zebrafish neuromasts (*sox2*, *eya1*, *eya4*), and candidates from the knifefish dataset including transcription factor genes (e.g., *klf5a*, *msx2b*) and a calcium-dependent cysteine proteinase subunit gene (*capns1*) that was highly enriched in the knifefish dataset. Shared gene expression supports a close developmental (and potentially evolutionary) relationship between neuromasts and ampullary organs.

Differential expression between neuromasts and ampullary organs was also seen for a few genes. The lab had previously found that one of the two catfish paralogs of the hair cell-specific marker otoferlin (*otofa*) was expressed in ampullary organs but not neuromasts (unpublished data). In this project, expression of *otofb* was found to be restricted to neuromasts. Otoferlin is a transmembrane protein required for synaptic exocytosis specifically in hair cells. Taken together, these data suggest an intriguing subfunctionalisation of the two *otof* paralogs that may be important for the differentiation of electroreceptors versus hair cells.

The transcription factor gene *foxe1* (also from the knifefish dataset) was expressed in ampullary organs but not neuromasts, potentially giving insight into the specification of teleost electroreceptors versus hair cells. Furthermore, the expression in catfish ampullary organs of candidate genes from the knifefish dataset may support the hypothesis that ampullary organs evolved in the common ancestor of knifefishes and catfishes.

Overall, these results shed new light on the development and evolution of electroreceptors in teleost fishes.

Experience gained: From this project, I have learned various experimental techniques which are highly applicable in many other studies in cell and developmental biology, from *in situ* hybridisation and immunohistochemistry to microscopy and imaging. I also gained key skills in routine molecular biology such as gel electrophoresis and PCR for riboprobe synthesis. In addition to these techniques, I was very fortunate to have had a chance to attend Cambridge Morphogenesis Symposium held in Cambridge, in September 2023. The project ended with an oral presentation in which I presented the findings of my project to the lab members. I will also prepare a poster to present my data at the AS Winter Meeting in January 2024. Overall, it was a valuable and fulfilling experience in real-world science.

Please state which Society Winter or Summer Meeting the student is intending to present his/her poster at:

The Anatomical Society Winter Meeting 2024 (January 2024, University of Liverpool)

Proposed Poster Submission Details (within 12 months of the completion of the project) for an AS Winter/ Summer Meeting – (no more than 300 words)

[This poster abstract has been submitted for the AS Winter Meeting in January 2024.]

Studying the development and evolution of teleost electroreceptors using the channel catfish, *Ictalurus punctatus*.

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The lateral line system of fishes and amphibians consists of mechanosensory and electrosensory divisions. Lines of neuromasts in the skin contain mechanosensory hair cells (very similar to vestibular inner ear hair cells) that detect local water movement. Many non-teleost fishes and amphibians also have electrosensory ampullary organs on the head: these sense weak, low-frequency electric fields in water and are primarily used for hunting. Previous studies in a non-teleost bony fish (paddlefish) and a cartilaginous fish (skate) have shown that ampullary organs, like neuromasts, develop from embryonic lateral line placodes and suggest that non-teleost electroreceptors evolved via the modification of lateral line hair cells. Electroreception was lost in the bony fish lineage leading to teleosts, but a few teleost groups are electroreceptive, including the Mormyrids (elephantfishes), Gymnotiformes (knifefishes) and Siluriformes (catfishes). Teleost electroreceptors are physiologically different from non-teleost electroreceptors and are thought to have evolved independently, also via modification of neuromast hair cells. To test this hypothesis, we studied the expression of candidate genes in channel catfish (*Ictalurus punctatus*) embryos and larvae using wholemount *in situ* hybridisation and immunostaining. Some candidates were selected from a putatively electroreceptor-enriched gene-set, arising from differential RNA-seq analysis of skin transcriptome data from the black ghost knifefish (*Apteronotus albifrons*), in a sister group to catfishes. Many candidate genes examined are expressed in both neuromasts and ampullary organs in catfish, including transcription factors (e.g., Sox2, Eya1, Klf5a, Msx2b) and a calcium-dependent cysteine proteinase subunit (Capns1) that was highly enriched in the knifefish dataset. Shared gene expression supports a close developmental (and potentially evolutionary) relationship between neuromasts and ampullary organs. The transcription factor Foxe1 (also from the knifefish dataset) was expressed in ampullary organs but not neuromasts, potentially giving insight into the specification of electroreceptors versus hair cells. Furthermore, the expression in catfish ampullary organs of candidate genes from the knifefish dataset may support the hypothesis that ampullary organs evolved in the common ancestor of knifefishes and catfishes. Overall, these results shed new light on the development and evolution of electroreceptors in teleost fishes.

Brief Resume of your Project's outcomes: **(no more than 200-250 words)**.
The title of your project and a brief 200-250 word description of the proposed/completed project. The description should include sufficient detail to be of general interest to a broad readership including scientists and non-specialists. Please also try to include 1-2 graphical images (minimum 75dpi). NB: Authors should NOT include sensitive material or data that they do not want disclosed at this time.

Studying the development of independently evolved electroreceptors in teleost fishes
Inner ear 'hair cells' convert mechanical stimuli (fluid movements inside the inner ear) into electrical signals for hearing and balance. Loss of hair cells leads to deafness and balance disorders, so understanding how hair cells develop, and how they regenerate in other animals, is the focus of much research. In fishes and aquatic-stage amphibians, virtually identical hair cells also exist in lateral line 'neuromasts' in the skin, distributed in lines over the head and trunk, which detect local water movement. In two different groups of teleost fishes - catfishes and knifefishes, including the electric eel; and the unrelated mormyroids - the lateral line system also includes electrosensory organs that respond to weak, low-frequency electric fields around other animals in water (used primarily for hunting). Electrosensory organs evolved independently in these lineages, most likely via the evolutionary modification of neuromasts. The aim of this project was to compare candidate gene expression in neuromasts versus electrosensory 'ampullary organs' in catfish embryos and larvae. Most genes examined, including some identified from a study in knifefish, were expressed (active) in both neuromasts and ampullary organs (Figure). This supports a close developmental relationship between neuromasts and ampullary organs, and potentially also an evolutionary relationship. A few genes were differentially expressed, either in neuromasts but not ampullary organs, or vice versa: this may give some insight into the molecular mechanisms that control the formation of hair cells versus electroreceptors. Overall, these results shed new light on the development and evolution of electroreceptors in teleost fishes.

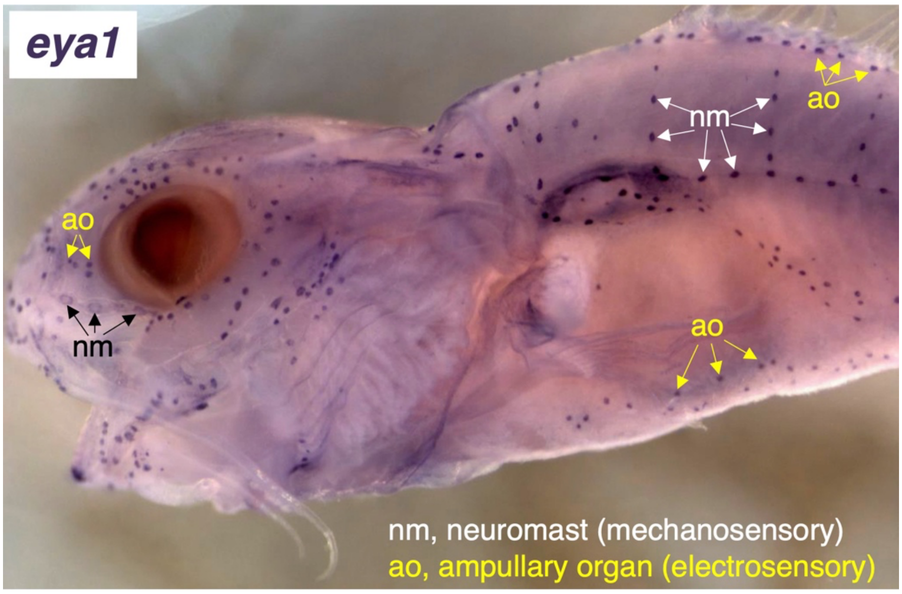

Figure: Expression of the transcription co-factor gene *eya1* in both mechanosensory neuromasts and electrosensory ampullary organs of a larval channel catfish (*Ictalurus punctatus*). Examples of neuromasts (nm) and ampullary organs (ao) are labelled. *Image credit*: Daiki Sugita.

Other comments: (no more than 300 words)

We are very grateful to the Anatomical Society for funding this project and for supporting Daiki's attendance at the AS Winter Meeting in January 2024.

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