Introduction
The enteric nervous system (ENS) is a network of nerves derived from neural crest cells that innervates the gut. Developmental abnormalities in the ENS can result in a number of gastrointestinal issues including Hirschsprung’s disease. Despite the amount of existing research on neural crest cell migration and differentiation, what specifically guides the migration and specialization of ENS precursors remains less understood. The chemokine receptor protein CXCR4 and its ligand CXCL12 play a role in axon guidance for the lateral line nerve and in the migration of sympathetic neuron precursors. The hypothesis behind this experiment is that these genes could also potentially play an important role in the development and functionality of the ENS.

Objectives
The goal of this project was to see if the ENS was able to (1) fully develop in the absence of CXCR4 or CXCL12 and (2) function properly in the absence of either of the two genes using zebrafish as a model.

Methods

Cross Fish
- Breed possible carriers of the mutant allele for either the ligand (CXCL12) or its receptor (CXCR4)

Lateral Line Staining
- Use DASPEI dye to visualize the lateral lines of the offspring
- Sort out embryos with an absent lateral line (mutants)

Immunohistochemistry
- Use HuC antibody to determine if enteric neurons are present in the gut of mutant embryos
- Compare mutant and wild-type embryos

Feeding Assay
- Give fluorescent food to both wild type and mutant embryos
- Assess the functionality of the enteric neurons by looking at how well embryos clear food from their gut

Results
Presence or absence of a lateral line was identified using a fluorescent dye, enabling embryos to be sorted into mutant and wild type groups. The mutant embryos are homozygous for either the mutated CXCL12 or CXCR4 allele and lack a fully formed lateral line. Immunohistochemistry to visualize the enteric neurons in the gut and found that the ENS was fully present in both mutant and wild type embryos for both genes. However, a feeding assay revealed that a majority of mutant embryos were unable to pass food as efficiently when compared to a majority of wild type embryos.

Conclusions
Immunohistochemical assays show that the ENS is able to develop fully even in the absence of the CXCR4 receptor or its ligand CXCL12. The fact that mutant embryos were less efficient in passing food during a feeding assay suggests that this protein pair could have a role in the functionality of the ENS. However, both of these genes are duplicated in the zebrafish genome. For example, CXCR4 has an CXCR4a copy and a CXCR4b copy. To test redundancy between these genes and how they contribute to the ENS, it may be necessary to test double mutants. The next step in this project would be to generate a line of fish from which double mutants could be acquired and to run the same series of tests on these embryos.