# Inner Ear Synaptic Changes in *Porichthys notatus* Supporting Seasonally Enhanced Acoustic Communication

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**Abstract**

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*Porichthys notatus*, commonly known as the plainfin midshipman fish, is a vocal fish found across the west coast of North America. Plainfin midshipman exhibit extreme seasonally dependent environmental alterations. Winter months are spent ~100m beneath the surface of the water; reproductively active summers occur in intertidal zones. he role of dopamine (DA) has been extensively studied in mammalian subjects, namely rodents. However, dopaminergic processes have previously remained understudied in ancient teleost fish. Type I Male *Porichthys notatus* rely on vocal signaling in the form of low frequency hums (100 Hz) to court gravid females during reproductively active summer seasons. Females thusly rely upon response to auditory stimuli and require significant neuroanatomical changes to compensate for vast changes in water depth. Conducted experimentation was hypothesized to show increased levels of dopaminergic processes in summer. Results showed an increase in count, volume, and proximity of DA terminals to regenerative hair cells in winter females, demonstrating a repressive aspect of the neuroreceptor. Moreover, hypothesized results included quantity and contact area of synaptic surfaces to increase in summer females, with a similar increase in darkened hair cell membrane ribbons. Experimentation showed quantity of synaptic contacts was decreased in winter as was area of contact compared to that of summer, both in total area and that adjusted to account for volume per images within electron microscopic stack. A reciprocal trend was observed in ribbons, wherein quantity remained increased in summer, while total and adjusted volume were less than that of winter.

## 1. INTRODUCTION

The Plainfin Midshipman, *Porichthys notatus*, is a species of toadfish designation, most commonly residing on the western coast of North America between 58° N and 28°N latitude and 136°W and 112° W longitude [3]. During the winter months, *Porichthys notatus* live in water of approximately 100 m in depth, migrating to intertidal or estuary zones during the spring and summer months during the reproductive periods. The Plainfin Midshipman is a species of interest due to its distinct neurological differences exhibited between winter and summer months. During summer months in shallow waters, male individuals generally fall into one of two designations as characterized by mating behavior. To better receive and process such calls, plainfin midshipman females exhibit a unique alteration in the level of dopaminergic processes and chemicals in specific areas of the nervous system. Here, afferent and efferent terminals are in constant flux, adjusting the capability of female midshipman to perceive various frequencies. The study of the CNS unique model organisms such as *Porichthys notatus* is integral to the understanding of the evolution of current auditory anatomy in a variety of species. This study contributes the role of dopamine in the fluctuation between reproductive activity in female midshipman. Dopamine was previously linked to larger catecholaminergic activities. Yet, the presence of large sites of dopamine transmission in Octavolateralis Efferent nucleus (OE) and Saccular Epithelium (SE) regions, often near afferent and efferent terminals, is poorly understood.

Figure 1—**Eggs of *Porichtys notatus***
Shown above is an overturned Type I male nest underneath a rock and resulting female eggs in the intertidal zone. The male will guard the nest, containing several hundred eggs. [3]

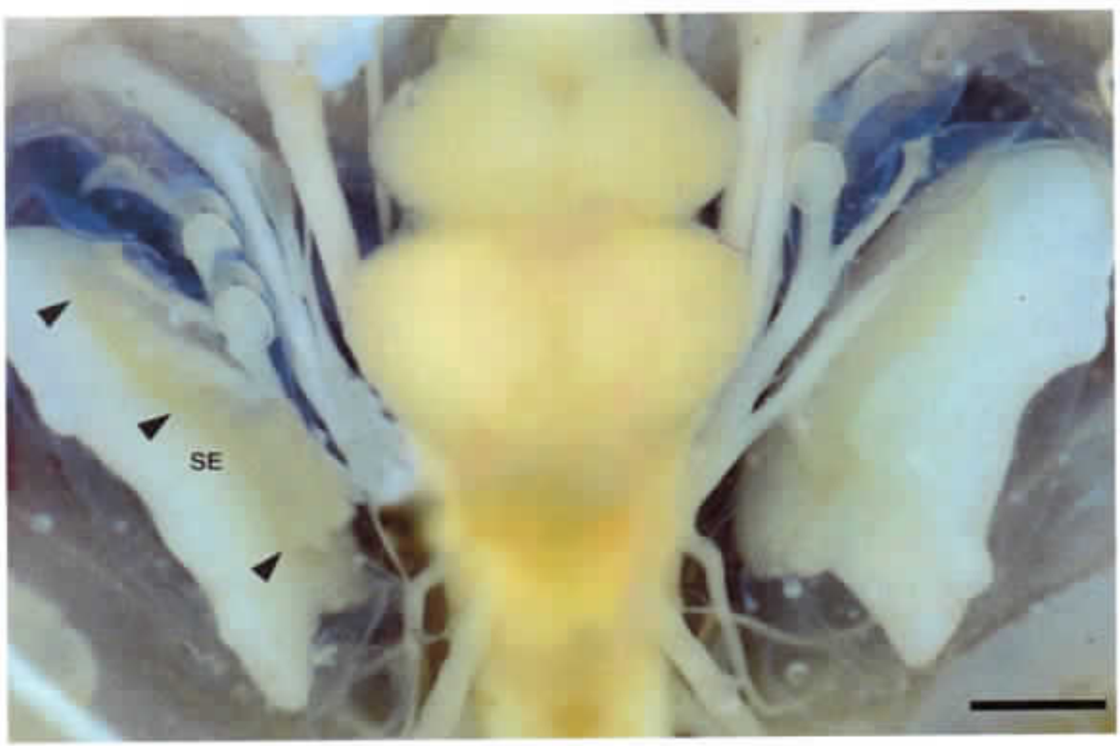


Figure 2—**Neuroanatomy of *Porichtys notatus***
Photograph depiction of plainfin midshipman brain from dorsal view. Shown on either side of brain are inner ear Saccular Epithelium (SE), with Octavolateralis Efferent nucleus (OE) shown at hindbrain. Stimuli enter through SE and are later processed following synaptic transportation. [Student Modified from Perelmuter, 2017]

This process begins through the reception of a Type I male hum through low frequency vibration travelling across the mud of the intertidal zone. Stimuli is passed through the SE to be processed through the hindbrain, most notably the OE. The processes occurring in the SE are of vital importance and are heavily reliant upon the regenerative nature of Midshipman hair cells. Here, signals are allowed cross between synapses in both the afferent and efferent directions. The role of dopamine (DA) in such a process has remained widely unknown in such processes. It is with great distinction that such a period of activity be compared to that of winter months, where females are reproductively dormant.

While reproductively active only once annually, female *Porcihthys notatus* depend upon the ability to receive and properly respond to deep hums of Type I Male Midshipman, or lack thereof from Type II Males. Type I Males are significantly larger than female and demonstrate an extremely low gonad-to-body ratios. Type I Males tend to build nests under the protection of heavy rocks, from which they court potential mates by emitting deep hums lasting up to 1 hour in length, of a steady frequency and volume (100 Hz). Agnostic growls are also used for combative purposes when defending a den from other Type I males.

In contrast, Type II Males are roughly 50% less massive, and reach sexual maturity around six months earlier than Type I Males. Rather than excavate nests, Type II males compensate for their small size by spraying satellite sperm in the general vicinity of a desired female, attempting fertilization. Type II Males have a gonad-to-body ratio 9 times that of Type I Males and dedicate 15% of their body weight to sexual organs.

The purpose of the experimentation at hand was to elucidate the effect quantity and area of synaptic surfaces exhibit on dopaminergic functioning in seasonal variance of acoustic communication capabilities in the vocal fish *Porichthys notatus.*

## 2. MATERIALS AND METHODS

*(Note that items marked with an asterisk (* or 2A) were completed by qualified research personnel including but not limited to associate professor and/or graduate students at CUNY Brooklyn College Department of Biology)*

### 2A. CAPTURE AND ACCLIMATION OF SPECIMEN*

Specimen (n = 12) *Porichthys notatus* were captured according to season, wherein winter midshipman (n = 6) were females captured during season by use of otter trowel at low depths. Gravid females (n = 6) were captured during summer season by hand through overturning male nests. Each were stored safely in tank room at CUNY Brooklyn College until experimentation began at which point the specimen were euthanized according to IACUC standards performed by qualified research personnel. Saccular Epithelium was then removed from dissected brain and stored until serially sectioned to a thickness of 60 nm. Electron microscopic images were taken and processed at which point they were transferred into the possession of student researcher as raw image files.

### 2B. IMAGE PROCESSING

Electron Microscopy was performed such that each given frame contained four (4) equal quadrants within field of view, following clockwise movement pattern. Process repeated first for each specimen and hereafter for any necessary duplicate slides.

Student researcher used open sourced FIJI Image Analysis Software in tandem with TrakEM2 ImageJ plugin. Quadrants within field of view were digitally stitched together to create cohesive stack of images. Five (5) such stacks were created for each animal (n = 12), each with an average of 14 images per stack.

These digitally processed images were then annotated with the student researcher identifying proper structures and subsequently tracing such. This allowed for the extraction of required data points, including volume, surface area, and quantity.

### 2C. DATA ANALYSIS

In order to properly execute accurate and relevant data measurements, a script was written in the Jython programming language. Output contained synaptic surface identification number, Average Smoothed surface of contact with hair cell, and the number of images in each stack such a process was observed. Ribbon measurements included identification number, volume, and number of images in which it was observed. Stacks were then manually analyzed to pair synaptic surfaces with their corresponding hair cells.

This output was then transferred into a workable excel spreadsheet and formatted for proper analysis by use of RStudio v1.2.1335 wherein analyses were performed including ANOVA, MANOVA, Student's t-test, and correlation. P values $< 0.05$ were considered to be significant.

### 2D. HEALTH RISK AND SAFETY

Student researcher did not in any way directly interact with specimen, neither dead no alive. In accordance with IACUC and ISEF regulations, all euthanization, dissection and serial sectioning of tissue were performed under proper conditions by qualified research personnel and did not in any way involve the student researcher.

## 3. RESULTS

### 3.1 Repressive Role of Dopamine

Results for the role of Dopamine (DA) in the repression of stimuli reception and response were additionally discussed in collaborative paper not yet in publication at CUNY Brooklyn College. These collaborative results demonstrated that DA is found in significantly greater quantities and volumes as observed in reproductively dormant female midshipman. Additionally, efferent DA terminals were found to have direct contact with adjacent hair cells at a significantly greater rate than those found in gravid females.

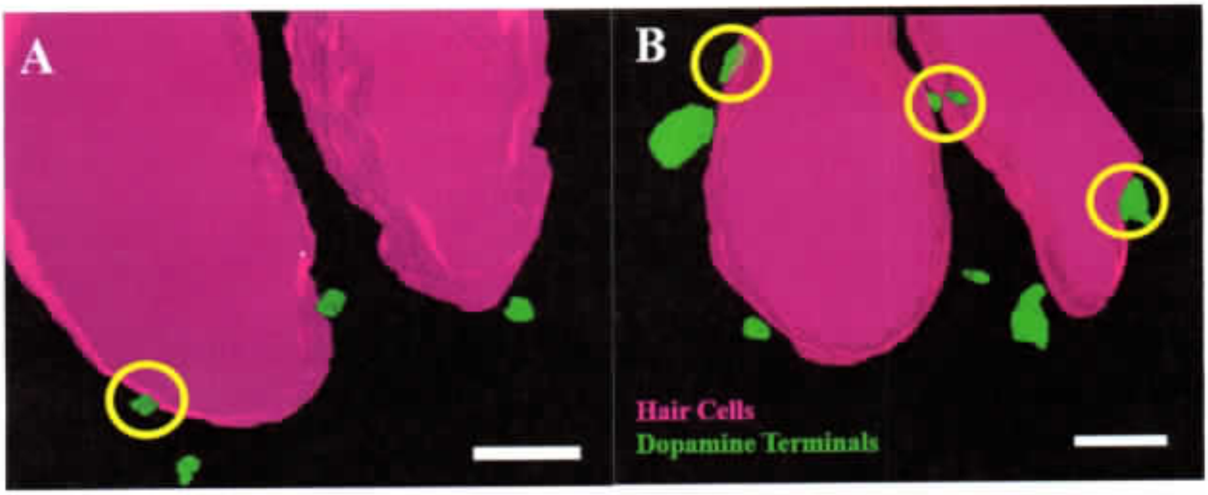


Figure 3 – **Repressive Role of Dopamine in 3D Render of Hair Cell**

Significant seasonal difference between both the size and the quantity of dopamine terminals in SE of midshipman between reproductive summers and dormant winters. Statistically significant difference observed between quantity of dopamine terminals found directly contacting hair cells in the SE between summer and winter. Scale Bar = 1 μm

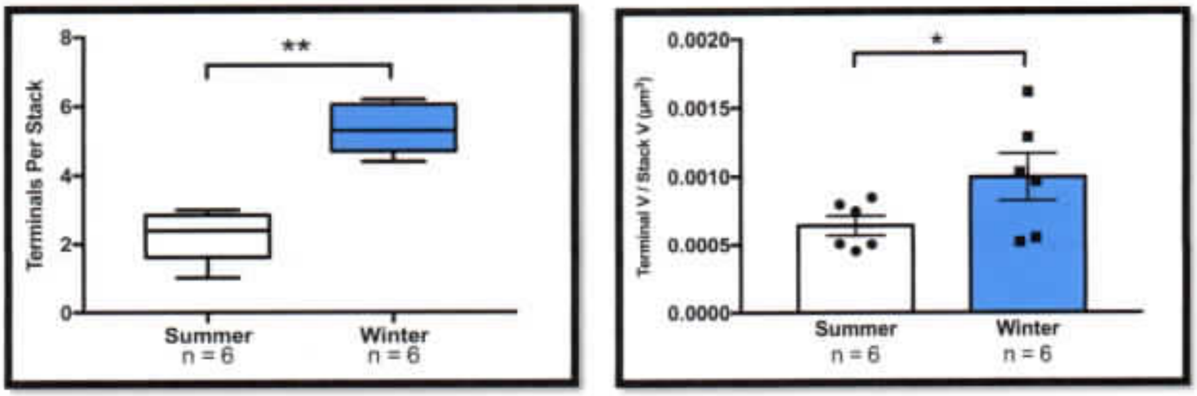


Figure 4 – **Statistical Visualization of Count and Volume of Dopamine**

Significantly more dopamine terminals are found in winter ($p = 0.0049$). Dopamine terminals are also significantly larger in winter than they are in summer ($p = 0.0435$)

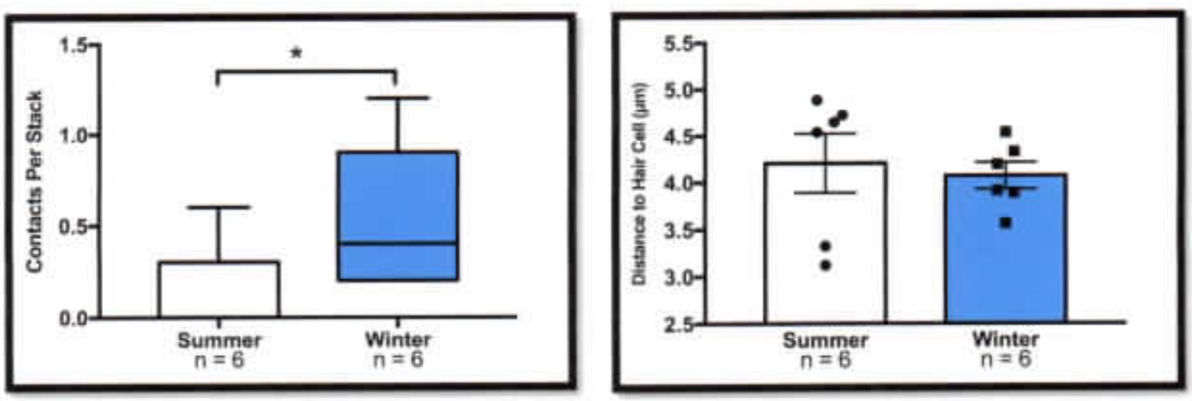


Figure 5 – **Statistical Visualization of Contacting Dopamine & Distance to Hair Cell**

Significantly more dopamine contacts are found in winter ($p = 0.0300$). However, the overall distance to Hair Cells from a given dopaminergic efferent terminal is not statistically significant ($p > 0.05$).

### 3.2 Seasonal Changes in Afferent Ribbons within Hair Cells

Results of experimentation revealed a slight decrease in afferent ribbon count from summer to winter females. Despite summer females demonstrating an overall larger count of ribbons, the volume of such was significantly smaller than that of winter females. This was shown true for both raw volumes per image stack, as well as those adjusted to include an average volume per image within a given stack.

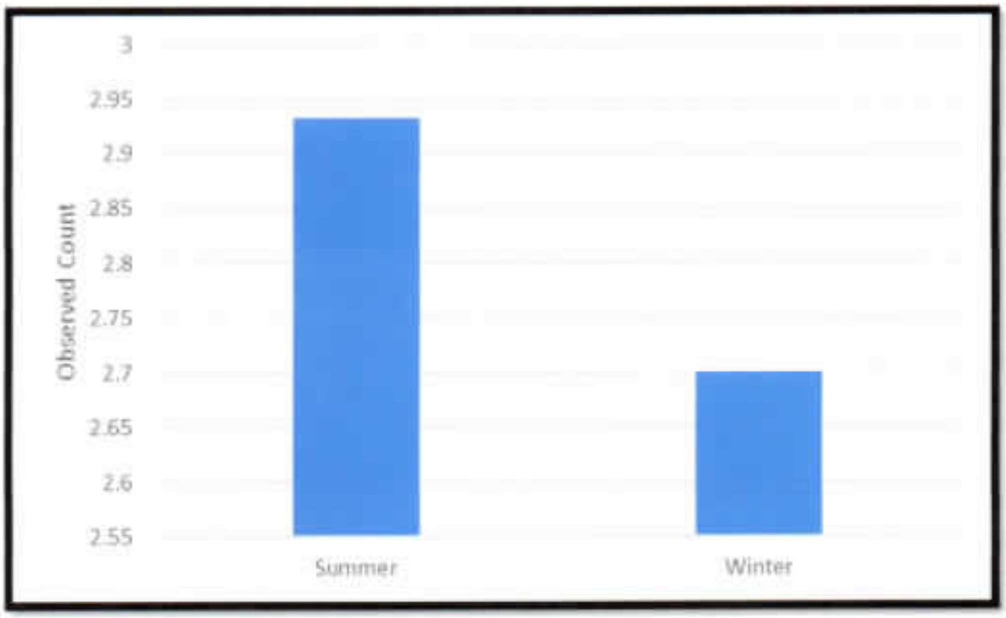


Figure 6 – **Differences in Quantity of Ribbons Between Seasons**

Count of ribbons between summer and winter months were shown to vary slightly. Average count of summer ribbons was equivalent to 2.93 per female, and only 2.70 per female in winter.

Figure 7 – **Seasonal Differences in Afferent Ribbon Volume**

Both raw and adjusted volumes demonstrated winter females exhibited a significant increase in volume of areas of darkened membranes than those in summer. ($p = 1.1E\text{-}04$, $p = 1.8E\text{-}06$)

### 3.3 Seasonal Changes in Synaptic Surfaces

Yielded results demonstrated the reciprocal changes in synaptic surface contact areas as compared to that observed above in afferent ribbons. Synaptic surfaces were measured as the direct contact area of an afferent synapse directly opposing that of ribbons. Furthermore, measured synaptic surfaces revealed the significant increase in contact area during summer months associated with a decrease in afferent ribbon volume. Once again, this held true for both raw and adjusted surface contact measurements.

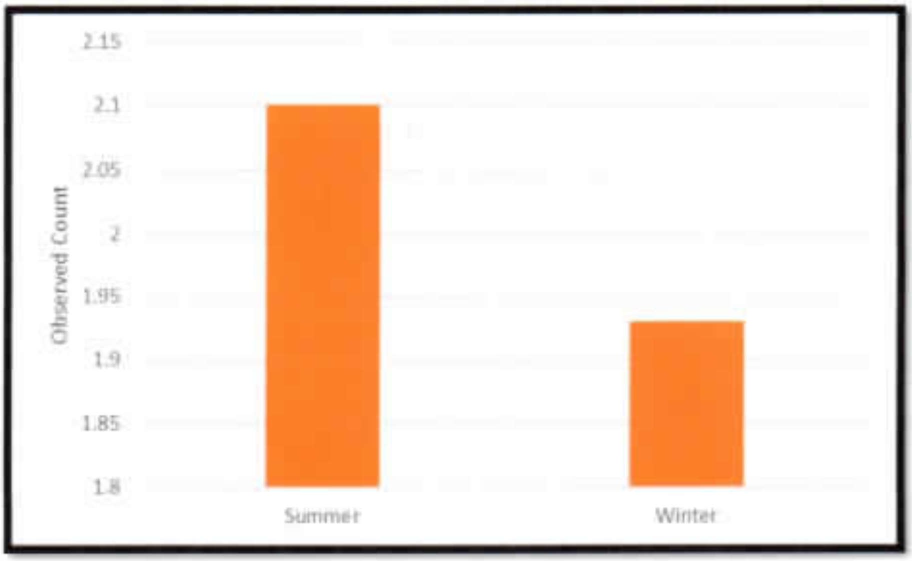


Figure 8 – **Differences in Quantity of Synaptic Contacts Between Seasons**

Observed count of synaptic contacts with adjacent hair cells was demonstrated to be larger during summer months, 2.10 per female, as compared to 1.93 during winter months.


Figure 9 – **Differences in Synaptic Contact Area**

Adjusted synaptic contact areas demonstrated summer females exhibited a significant increase in volume of areas of darkened membranes than those in winter, opposite of what was previously observed in afferent ribbons. ($p = 5.7E\text{-}04$)

## 4. DISCUSSION AND CONCLUSION

The objective of this experimentation was to elucidate the extent to which synaptic changes adjusted the neurobiology of *Porichthys notatus* to accommodate seasonal changes in environment and reproductive activity. This experimentation included 12 specimens and 5 stacks of images for each animal, which were categorized by the season in which they had been captured and observed.

Expected results were those to have been directly representing the severe alteration in surrounding environment and reproductive objective which is observed in summer female plainfin midshipman. Yielded results demonstrated the extent to which the anatomical structure of the SE is altered to accommodate such changes.

When observed during winter months, reproductively dormant females were shown to have a distinct morphology characterized by large quantities of dopaminergic efferent structures, each with large volumes. These winter dopamine structures also tended to make direct contact with adjacent hair cells. As compared to the SE of gravid summer females, the differences were distinct. In summer, females exhibit a lesser quantity of dopaminergic efferent structures, each of those with a significantly lesser volume. In direct contrast to their winter counterparts, summer dopamine structures rarely made direct contact with hair cells. This sheds light on the ability of dopamine to exhibit functions other than the accepted ability to protect against loud sounds. Furthermore, dopamine has now been shown to have a direct suppressive effect on the ability of female midshipman to receive and respond to low frequency hums. This may be due to the lack of necessity to do so, considering the lack of reproductive activity at this time, as well as the larger ocean depths.

Female plainfin midshipman also developed varying afferent ribbon and synaptic surface contact activity levels between seasons. In winter, females were shown to have contain fewer ribbons, each with large volume located directly adjacent to a smaller formation of synaptic surface contacts with the given hair cell. The count of synaptic surface contacts was also minimal. This combination would damage the ability to accurately and efficiently transport stimuli to the hindbrain.

Once observed in summer months, a direct parallel was observed. Gravid females tended to have a larger quantity of both ribbons and of synaptic surface contacts. Each ribbon was of lesser volume than was observed in winter. Synaptic surface contacts, however, were shown to have been both greater in count and size. This allows for the streamlined communication of information, as each ribbon is required to carry less synaptic signaling and contains the added benefit of a large surface area upon which such information may be carried.

It is apparent that the distinct seasonal changes in morphology for female *Porichthys notatus* serve the direct purpose to quickly and efficiently receive and respond to Type I male calls in order to accurately and reliably choose a given suitor.

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