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BIOLUMINESCENT FISH, BACTERIA, AND EXPERIENTIAL LEARNING

A thesis submitted to
Regis College
The Honors Program
in partial fulfillment of the requirements
for Graduation with Honors

by

Ryan Barton

May 2016

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Introduction

As my freshman year at Regis came to a close, I decided to get involved in biological research. I have always had an interest in laboratory work for the opportunities of exploration and discovery it offers. Furthermore, I saw research as an excellent opportunity to put into action what I had learned in class. I wanted to act on my learning, and engage with the scientific world beyond my textbooks. Fortunately, a great opportunity was presented, and I began conducting research on bioluminescent fish with Dr. Ghedotti, a project that grabbed my attention immediately. Through this research and all the other laboratory experiences I have had at Regis, I began to see how strongly reinforced my science education had become.

I place a large emphasis on experience in education because this is the process by which we learn anything in life. Our lives are a collection of stories and events that coalesce to form our individual characters. We continually take in information and occurrences, ways to see the world, to think about issues, and interact with others. The passive collection of these experiences is only the first step, however. The synthesis of our daily experiences provides each person the knowledge of how to live better, to make choices that align with their values, and to more clearly conceive the world as it is. Our consciousness is continually developing as we are shaped by the events around us, which is very important because this ultimately alters how we choose to engage with the people we encounter.

Therefore, I place a very high value on the opportunities that I have in life and how I react to them. As a biology student, I value very much the opportunity to engage with my learning in the unique setting of the laboratory due to the maturity of scientific understanding that research offers. As a citizen concerned about the development of my country, I value my ability to talk to my compatriots about their views on education, healthcare, and the economy of our country. I may not agree with what I hear, but to share in the experience of a healthy debate is a wonderful opportunity as it presents mindsets I may have not previously considered and novel ways of synthesizing ideas. Encountering the unknown and unfamiliar rarely does not evoke complacency. Rather, our experiences challenge us, excite us, and most of all engage us with the reality in which we live.

After working with Dr. Ghedotti on the evolution of light organs of a bioluminescent fish, I decided I wanted to branch out and try my hand at a project I had developed myself. The opportunity to do so was made possible to me by the support of Dr. Ghedotti as well as an undergraduate research grant offered by Regis. I realized that I had all the tools I would need to conduct my own research project, thus I took the opportunity and began my study of bioluminescent bacteria that are housed within certain species of marine fish. I was interested in the genetics of these bacteria, with a focus on the communication genes present in their genomes. While I did not support my hypothesis about these communication genes, I was rewarded with the experience of learning, developing, and altering laboratory procedures I had learned about in the

classroom to answer my own questions. I learned a great deal in these research projects, and I greatly value the practical nature of the work I did.

Evolution and genetics greatly impact the development of deep-sea fish and bacterial bioluminescence. Furthermore, morphology is fundamental to understanding the relationship between form and function that is so essential to understanding biology and, more specifically, evolution. In my first chapter, I explore the anatomical evolution of two bioluminescent organs in the deep-sea fishes *Lestrolepis* and *Lestidium*. In my second chapter, I explore the evolution of genes associated with bioluminescence in bacteria housed by deep-sea fish, specifically looking for quorum quenching genes in bacteria from the genera *Photobacterium* and *Aliivibrio*. Finally, in my third chapter, I reflect on how this body of research has led me to reflect on how learning by experience has been such a valuable part of my education here at Regis, and how I see experiential learning to be very important to the development of self.

Chapter 1 – Characterization of Anatomy, Morphology, and Evolution of Lestidiid Light Organs

Introduction

Bioluminescence is an intriguing biological phenomenon. Essentially, it consists of living organisms that are able to produce light and emit it for various purposes. The reaction that produces light is a chemical one that transfers energy from a reaction into light energy. Animals, bacteria, fungi, and plants across many ecosystems accomplish bioluminescence. My interests are in bioluminescent fish.

The actual mechanism by which organisms produce light relies on a straightforward conversion of energy. An enzyme known as luciferase chemically alters its substrate compound, generally referred to as luciferin (Herring 2002). The alteration that occurs is an oxidation of the luciferin molecule, which brings it to a higher energy state. The energized luciferin then returns to a stable energy level, and the energy that is given off in the process is emitted as light (light of a frequency equivalent to the difference in energy), thus an organism is able to transform chemical energy into light energy (Herring 2002).

The luciferases and luciferins of bioluminescent fishes likely evolved multiple times independently from a similar precursor system. The luciferase-luciferin system likely is a co-opted antioxidant system, which all organisms exposed to oxidative stress

must possess in order to withstand harmful free radical compounds. Now, however, the enzymes and proteins carry out a variation of the same reaction, yet light is the product, rather than mere neutralization of dangerous free radicals (Rees et al., 1998).

The emission of light by a marine fish may be conducted endogenously, within the tissues of the organisms' light organ, or exogenously, via formation of symbiotic relationships with bioluminescent bacteria that are housed within the fish and emit light (Herring, 2002). In the case of endogenous bioluminescence, the tissue conducting the bioluminescence may be derived from a multitude of tissues (Haddock et al., 2010). Exogenous bioluminescence often relies on diverticula and extensions of the digestive tract (e.g., coelom) to house symbiotic bacteria derived from the environment (Wassersug & Johnson, 1975).

Bioluminescence in deep-sea fish evolves or is maintained for many reasons. Light organs and emissions may act to offensively or defensively benefit the luminescent species. Light organs may act to lure prey, distract predators, or camouflage the fish, the latter constituting the type of bioluminescence focused on henceforth. The selective pressure of predator avoidance has led to the bioluminescence phenomenon of counterillumination evolving in many groups of mesopelagic fishes (Herring, 2002). Fish in this region of the ocean live in a habitat with moderate downwelling sunlight, thus giving the region the nickname the "twilight zone." Here, fish are susceptible to predators beneath them, as the weak downwelling sunlight forms a readily visible silhouette. Counterillumination eliminates this silhouette by emitting light on the underside (ventral

side) of the fish, which allows the fish to obscure its silhouette to any predators swimming below (Lawry, 1974; Young & Roper, 1977).

Oftentimes, these fish are capable of matching the intensity of their ventral bioluminescence with the downwelling sunlight, allowing for better camouflage. Photophores (the tissue conducting light-emitting reactions) located above or in front of the eyes allow for the current light conditions to be detected and subsequently matched by ventral bioluminescent organs (Young & Roper, 1977). Thus, counterillumination represents a very complex physiological phenomenon that offers an adaptive advantage to those species that produce it.

One such group of counterilluminating fishes is the naked barracudinas, which belong to the family Lestidiidae that includes the genera *Lestrolepis* and *Lestidium*. Lestidiid fishes inhabit the mesopelagic zone where ventral counterilluminating bioluminescence is especially beneficial. Species belonging to this clade are the subject of investigation for this study concerning the morphology and evolution of bioluminescent light organs of deep-sea marine fish.

Haneda (1958) first reported on the bioluminescence of the naked barracudinas (Order Aulopifromes, Family Lestidiidae) five years after the initial description of the family (Harry, 1953). The light organs of *Lestrolepis* sp. and *Lestidium prolixum* proved to be “self-luminous” (i.e., endogenous luminescence and not driven by bacterial symbionts), bi-lobed, yellow organs running the ventral length of the fishes (Haneda, 1958). Furthermore, the light organ in these fishes is covered ventrally by transparent muscle tissue (Haneda, 1964). In other species, the transparent muscle tissue functions as

a window and lens through which emitted light passes, as it intensifies the dispersal of emitted light to ensure maximum efficacy (Herring, 2002). Also, *Benthalbella infans* (Family Scopelarchidae) was identified as possessing a ventral luminescent organ derived from skeletal muscle and sharing structural similarities with light organs of the naked barracudinas (Johnston & Herring, 1985). Although the presence of the ventral bioluminescent organ in *Lestrolepis* and *Lestidium* is well documented (Haneda, 1958; Haneda, 1964), the organ or tissues from which it was evolutionarily derived remains unclear beyond the suggestion by Johnston and Herring (1985) that it is derived from skeletal muscle. In addition, *Lestrolepis* individuals have a distinct dark spot anterior to the eye (Harry, 1953) that has yet to be examined anatomically and may be associated with bioluminescence. This study seeks to identify the anatomical composition and possible origins of both structures.

Methods

Gross Dissection

Specimens used were ethanol-preserved museum specimens (on loan from the Field Museum of Natural History, courtesy of W. C. Smith and C. McMahon). I worked with M. Ghedotti to perform dissections on the specimens available. Dissections were performed by making right ventral incisions just anterior to the anus and cutting anteriorly until reaching the isthmus, at which point incisions were made around the pelvic girdle so as to avoid damaging the bone structure in this region. While examining the coelom of the specimens, the falciform mesentery (the ligament attaching the liver to

the body wall) of *Lestrolepis* and *Lestidium* specimens had to be removed in order to gain visual access to the viscera. The specimens were examined and documented using a Leica MZ 12.5 stereomicroscope with an attached Q Imaging MicroPublisher 5.0 RTV photodocumentation system.

Histological Analysis of Bioluminescent Organ

The histological analysis of light organs entailed making very thin sections of the specimens of interest. A portion of the tissue was removed, embedded in medium of choice (LVN or paraffin in this study), which ultimately allows for these media to replace water in the tissue and provide the support necessary to permit thin slicing. The specimens were then cut into thin sections using a microtome and placed on microscope slides. Then we used stains to identify certain tissue types and organ structures.

The histological analysis of portions of *Lestrolepis japonica* and *Lestidiops jayakari* was conducted by fixing the samples in both low viscosity nitrocellulose (LVN) as well as paraffin. I was not a part of the very long LVN specimen preparation, yet I was involved in examining the sections. Regardless, in order to prepare the LVN sections, specimens were prepared by decalcification for a week (removal of calcium ions from bones so that sectioning is made easier), then by dehydration in ethanol (remove water to allow for replacement with a hard matrix that supports sectioning). Specimens were then infiltrated with nitrocellulose via a series of increasing nitrocellulose concentrations. After drying the specimens, they were sectioned on a sledge microtome at 100 μm (Humason, 1979). Sections were stained using a Picro-Ponceau S (PP) staining protocol

(Humason, 1979), and then mounted on slides with Permount.

Paraffin sections were prepared by first dehydrating the specimens in ethanol. The specimens were placed in increasing concentrations of ethanol up to 100%, and then cleared in xylene (an organic solvent). The specimens were then embedded in paraffin, a wax, by immersing the specimens in two subsequent liquid paraffin baths for 8 hours each, then situating the specimen in a solid block of paraffin wax. The specimens were sectioned at 10 μm using a rotary microtome, and the resulting sections were mounted on glass microscope slides. I stained the sections using Masson's trichrome (MT) stain protocol (Sheehan & Hrapchak, 1980; Bancroff & Stevens, 1982). MT allows for the differentiation between collagen, which stains blue, and smooth muscle, which remains pink. LVN and paraffin slides were examined and photographed with a Leica DM 2500 compound microscope and a Q Imaging MicroPublisher 5.0 RTV photodocumentation system.

Phylogenetic Analysis

In addition to the dissection and histological work we completed, M. P. Davis participated in our study by providing DNA sequence data and a phylogenetic analysis of the species studied to construct a phylogeny, which would eventually be overlaid with the anatomical data obtained. For the phylogenetic analysis, 18 species of fish from six families (Evermannellidae, Sudidae, Alepisauridae, Anotopteridae, Paralepididae, and Lestidiidae) within the order Aulopiformes were compared. An Evermannelid species (*Odontostomops normalops*, a sabertooth fish) functioned as the outgroup in the

phylogenetic analysis due to its position among aulopiform fishes (Davis & Feilitz, 2010). Eight genes were used: one mitochondrial (cytochrome oxidase I, 812 base pairs) and seven nuclear genes coding for various proteins (ectodermal-neural cortex 1-like gene, 845 bps; glycosyltransferase gene, 727 bps; myosin heavy chain 6 alpha gene, 759 bps; pleiomorphic adenoma protein-like two-like gene, 852 bps; ptr hypothetical protein, 765 bps; recombination activating gene 1, 1,452 bps; zic family member protein, 889 bps). The genes were aligned (process by which multiple genes are aligned to assess sequence homology, which allows for elucidation of shared evolutionary origins between these genes and ultimately the organisms themselves) using the program MAFFT v6.0 (Multiple Alignment using Faster Fourier Transform, a multiple sequence alignment program for comparison of gene sequences), run using the program's default parameters. A maximum-likelihood analysis was then conducted in order to estimate the most likely pattern of genetic evolution for each gene and each category of position within each gene. The models of molecular evolution, were selected using jMODELTEST v.2.1 (Posada, 2008), which assigned the most probable model of evolution for each of the eight compared genes (done using Akaike information criteria, a measure of the quality of the estimated models). Five maximum likelihood analyses were performed using the program GARLI v2.01 (Genetic Algorithm for Rapid Likelihood Inference) to generate the most likely phylogenetic tree based on the data in the context of the genetic models of evolution (Zwickl, 2006). The three with the highest likelihood scores were used for phylogenetic analysis.

Study Specimens

Specimens were obtained from various museum collections. All were preserved in ethanol and catalogued. Those with asterisks succeeding the museum catalogue numbers were used to perform histological analyses and gross dissections. The specimens used were as follows (catalogue number given, number in parentheses indicates number of specimens in lot, location given): Alepisauridae. *Alepisaurus ferox* MCZ 127309 (1) no data. *Omosudis loweii*: KU 38782 (1) North Atlantic Ocean, 39° 460 N, 67° 320 W. MCZ 163183 (1) North Atlantic Ocean, 39° 520 N, 67° 150 W. Anotopteridae. *Anotopterus pharo*: MCZ 148409 (1) no data. MCZ 164375 (1) North Atlantic Ocean, 39°500N, 67°270W. Evermannellidae. *Coccorella atlantica* MCZ 73021 (1) North Atlantic Ocean, 38° 240 N, 71° 80 W. *Evermannella balbo* MCZ 52329 (1) North Atlantic Ocean, 40° 320 N, 63° 470 W. *Odontostomops normalops* MCZ 127171 (1) North Atlantic Ocean, 8°560N, 46°380W. Lestidiidae. *Lestidiops jayakari*: FMNH 117867* (4) South Atlantic Ocean, 12°370S, 11°140E. *Lestidiops ringens*: SIO 79187 (2) South Atlantic Ocean, 12°370S, 11°140E. *Lestidium atlanticum*: KU 27946 (1) North Pacific Ocean. *Lestrolepis intermedia*: MCZ 91605 (5) North Atlantic Ocean, 35° 580 N, 74° 460 E. *Lestrolepis japonica*: FMNH 120671* (19) North Pacific Ocean, Taiwan Dong Gang Fish Market. *Macroparalepis brevis*: MCZ 68502 (1) North Atlantic Ocean, 23°040N, 45°100W. Paralepididae. *Arctozenus risso*: MCZ 95211 (1) North Atlantic Ocean, 42°470N, 69°580E. *Magnisudis atlantica*: VIMS 05991 (18) North Atlantic Ocean. *Paralepis coregonoides*: MCZ 158994 (1) North Atlantic Ocean, 39° 550 N, 67° 250 E. *Paralepis* cf. *brevirostris*: FMNH 85322 (9) North Pacific Ocean, 27°070N,

138°560E. Sudidae. *Sudis hyalina*: MCZ 43077 (1) Mediterranean Sea, Strait of Messina.

Results

Gross Dissection of Coelomic Viscera

Gross dissection revealed that the ventral bioluminescent organ is composed of hepatopancreatic tissue (combined liver and pancreatic tissue). All species examined exhibit a primary hepatopancreatic lobe located anteriorly, which extends to variable lengths among different species (Fig. 1. A, B). Among the fish examined outside of the family Lestidiidae, the hepatopancreatic tissue extends from the anterior portion posteriorly as two lobes dorsal to, and running along the length of, the intestine (Fig. 1. A). As for Lestiidid fishes, a similar structure is observed, yet the genus *Lestidiops* demonstrates a third lobe of hepatopancreatic tissue running posteriorly along the intestine between the intestine and the ventral body wall (i.e., ventral to the intestine; Fig. 1. B).

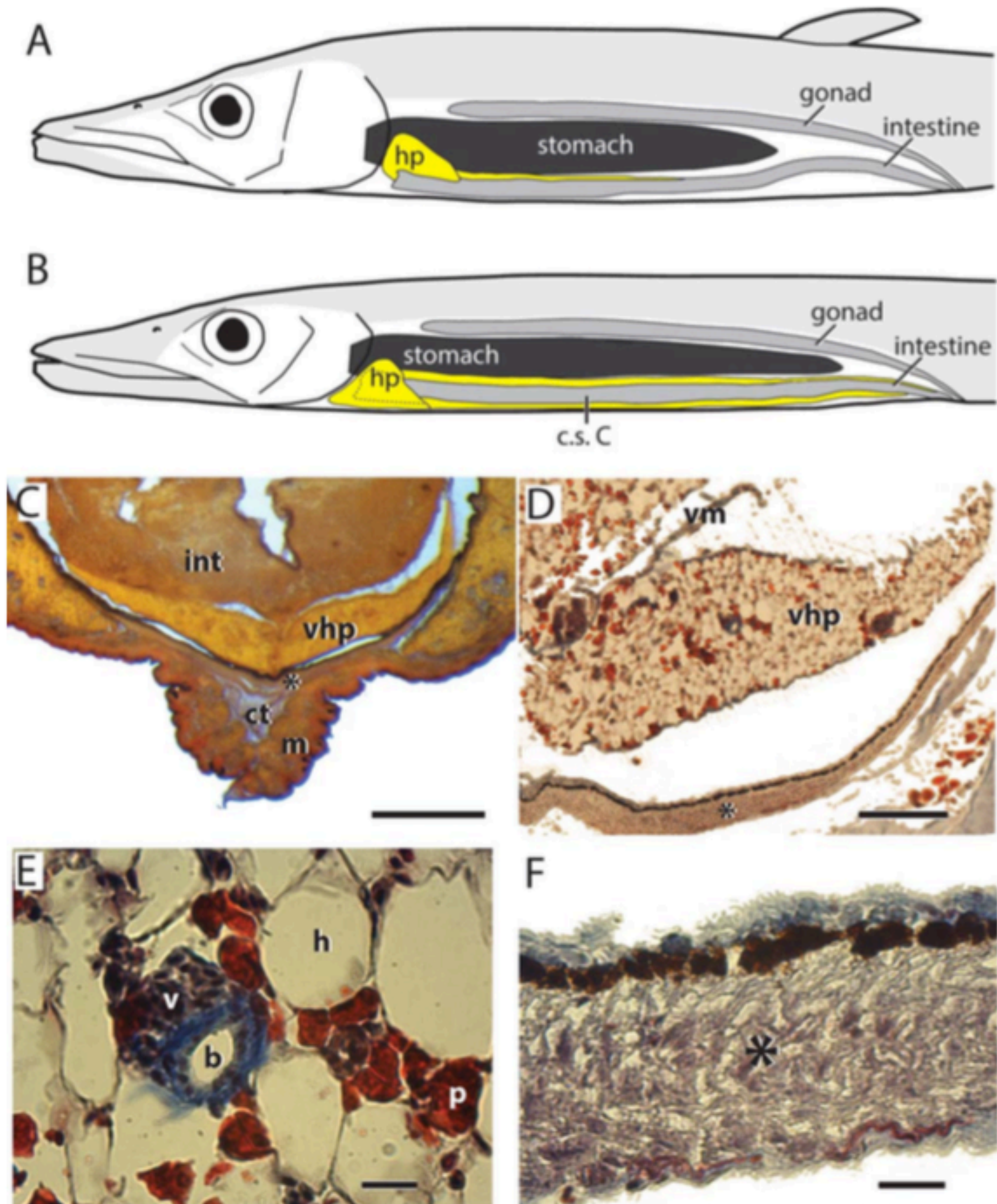

Fig. 1. Diagram of major viscera in (**A**) *Macroparalepis atlantica* and (**B**) *Lestidiops jayakari*, dashed line delineates intestine position. (**C**) 100 µm thin LVN cross section of *L. jayakari* ventral trunk region, PP. Bar = 1 mm. (**D**) 10 µm thin paraffin cross section of *L. jayakari* ventral bioluminescent organ and related tissues, MT. Red staining regions represent hepatopancreatic blood vessels. Bar = 200 µm. (**E**) 10 µm thin paraffin cross section of central region of **D**, demonstrating sinuses and related blood vasculature of hepatopancreatic tissue, MT. Bar = 20 µm. (**F**) 10 µm thin paraffin cross section of *L. jayakari* ventral peritoneum showing the pigmented layer with smooth muscle and connective tissue beneath, MT. Bar = 20 µm. Asterisk, ventral peritoneum; b, bile duct; c.s. C, region of section **C**; ct, connective tissue; h, hepatocyte; int, intestine; m, muscle tissue; p, pancreatic tissue; v, vein; vhp, ventral hepatopancreas; vm, ventral mesentery. (Ghedotti, Barton, Simons, & Davis, 2015).

The ventral lobe of the hepatopancreas extends to the middle of the coelom (body cavity) of *L. ringens* and to just anterior of the anus in *L. jayakari*. In *Lestidium* and *Lestrolepis*, both of which species display ventral bioluminescence, the ventral luminescent organ runs in the same position as the ventral lobe of the hepatopancreas, likewise associated with a contiguous ventral mesentery (Fig. 2A). However, the bioluminescent organs are differentiated from the hepatopancreas, as they are lighter in color (more yellow than orange) and are more delicate in structure in comparison to the ventral hepatopancreatic strand in other taxa.

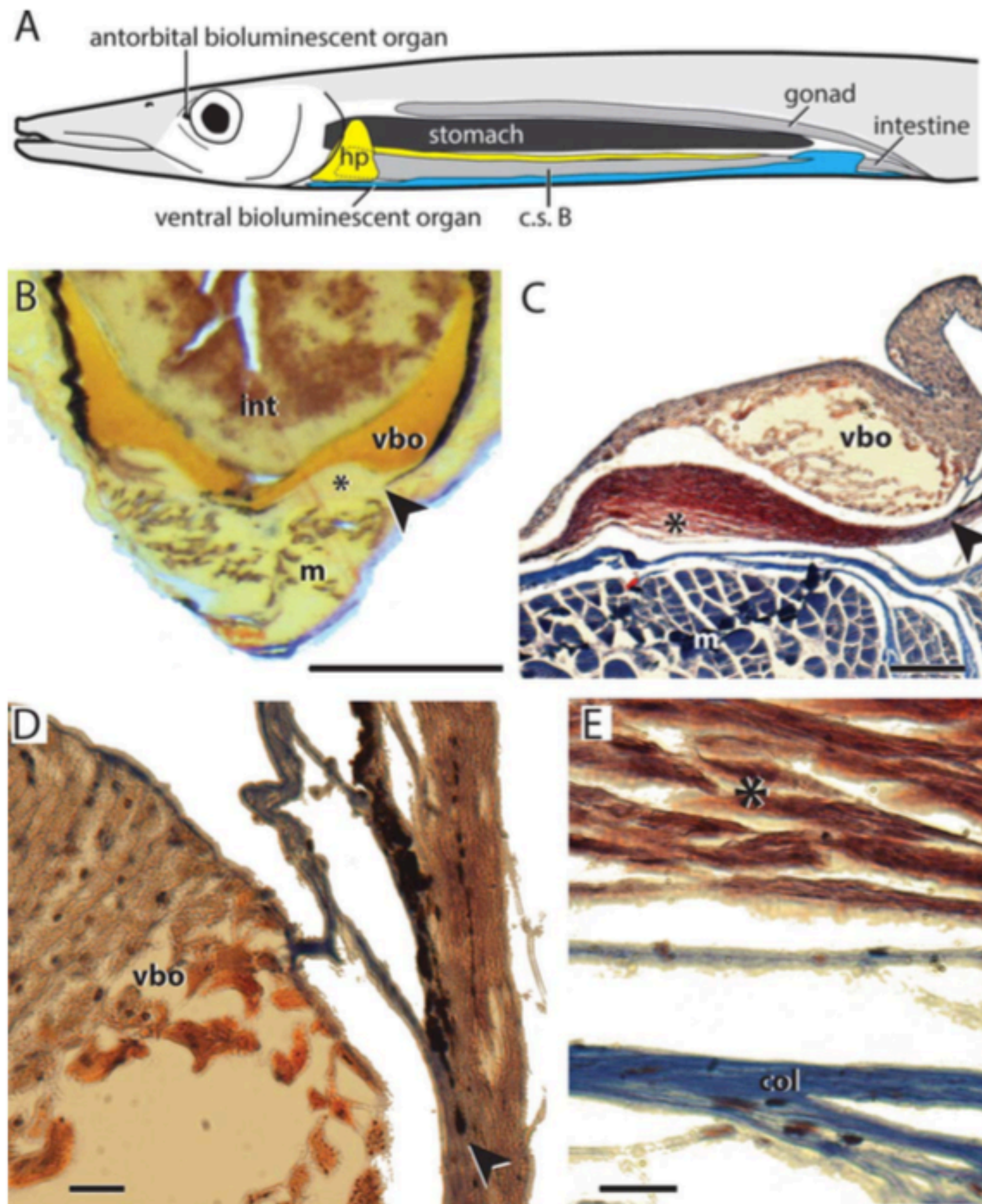

Fig. 2. The ventral bioluminescent organ in *Lestrolepis japonica*. (**A**) Diagram of major viscera in *L. japonica*, dashed line delineates intestine position. (**B**) 100 µm thin LVN cross section of ventral trunk, PP. Bar = 1 mm. (**C**) 10 µm thin paraffin cross section of the left ventral region of the bioluminescent organ, MT. Note sinuses present in central portion. Bar = 200 µm. (**D**) 10 µm thin paraffin cross section of central region of **C**, MT. This shows the transition from tightly packed cells to loose cells containing granular inclusions (likely photophores) surrounded by sinuses (indicative of hepatopancreatic tissue). Bar = 20 µm. (**E**)10 µm thin paraffin cross section of left peritoneal lens demonstrating smooth muscle composition as compared to associated connective tissue (collagen), MT. Bar = 20 µm. Arrow head, ventral-most extent of pigmented and reflective peritoneum; asterisk, thickened smooth muscle of ventral peritoneum; col, collagen; c.s. B, approximate position of section depicted in B; hp, hepatopancreas; int, intestine; vbo, ventral bioluminescent organ; m, skeletal muscle. (Ghedotti, Barton, Simons, & Davis, 2015).

Ventral Hepatopancreas and Bioluminescent Organ Histology

Upon histological analysis of the ventral hepatopancreatic and luminescent tissues, a great deal becomes apparent about the structure and morphology of these structures. In the non- luminescent species *Lestidiops jayakari*, the ventral lobes of the hepatopancreas extending posteriorly along the intestine are clearly composed of hepatocytes, pancreatic cells, and ducts that associate the tissue with the intestine (Fig. 1. C, D, E). Furthermore, a pigmented peritoneum completely surrounds the hepatopancreas (Fig. 1. F). With the cross-sections examined from *Lestrolepis japonica*, the ventral bioluminescent organ lacks such ducts as seen in *L. jayakari*, and it is less vascularized (indicating less function as a typical hepatopancreas; Fig. 2. C, D). The cells appear to possess granular inclusions dorsally (consistent with photophore construction) and sinuses between cells are apparent, which may indicate the hepatopancreatic origin of the bioluminescent organs (Fig. 2D). Furthermore, the otherwise pigmented peritoneum becomes transparent ventral to the luminescent organ, as it lacks melanocytes in this region. Between the ventral side of the luminescent organ and the body wall, this transparency continues, as the smooth muscle tissue beneath is highly ordered (Fig. 2. D, E).

Antorbital Light Organ

The antorbital spot (a pigmented spot lying in front of the eye) of *Lestrolepis japonica* appears to be rounded on its anterior and dorsal sides (front and top), with a

straight margin that extends on the rear side that extends downward as a pigmented border (Fig. 3. A). After histological analysis, the pigmented region of this structure appears to form a cup around a group of cells. These cells possess distinct nuclei and granular inclusions, which transition to more irregularly shaped cells in the middle of this cup-like structure. Posteriorly, this cup contains connective tissue associating the structure with the eye (Fig. 3. B, C). This structure is present only in individuals in the genus *Lestrolepis*. No other lestidiid fishes demonstrate such preorbital structures.

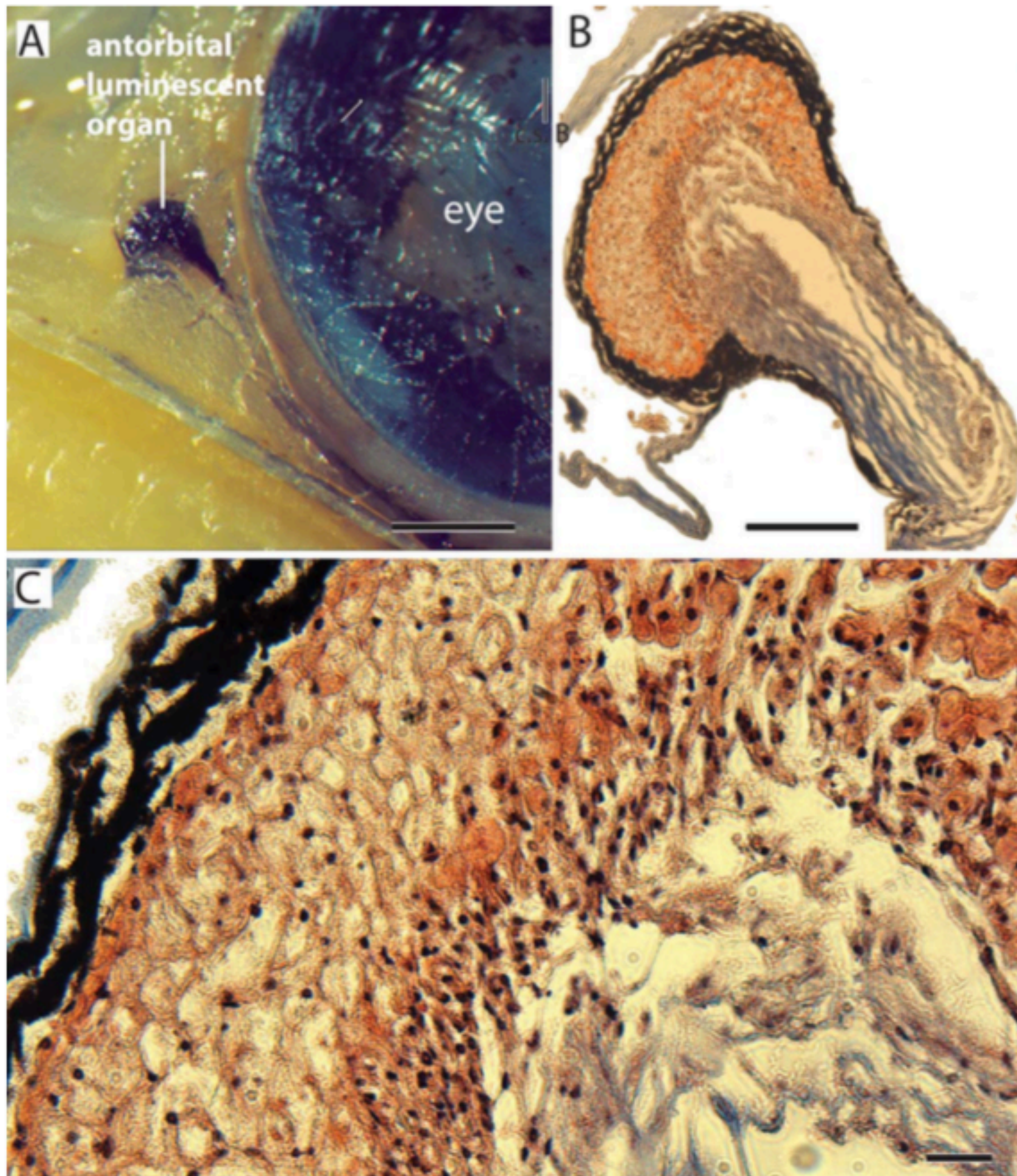

Fig. 3. Antorbital bioluminescent organ in *Lestrolepis japonica*. (**A**) Left lateral view of antorbital light organ in an alcohol-preserved specimen. Bar = 1 mm. (**B**) 10 µm thin longitudinal paraffin section of the left antorbital light organ showing pigmented connective tissue surrounding bioluminescent cells, and unpigmented closest to the eye. Bar = 200 µm. (**C**) 10 µm thin longitudinal paraffin section of the left antorbital light organ showing compact cells with granular inclusions transitioning into less compact and elongate cells. Bar = 20 µm. (Ghedotti, Barton, Simons, & Davis, 2015).

Phylogenetic Analysis

Additionally, the phylogenetic data compiled by Davis is informed by the anatomical and histological data. It can be seen that the bioluminescent species in the genera *Lestidiops* and *Lestrolepis* form a monophyletic group within the family Lestidiidae (Fig. 4).

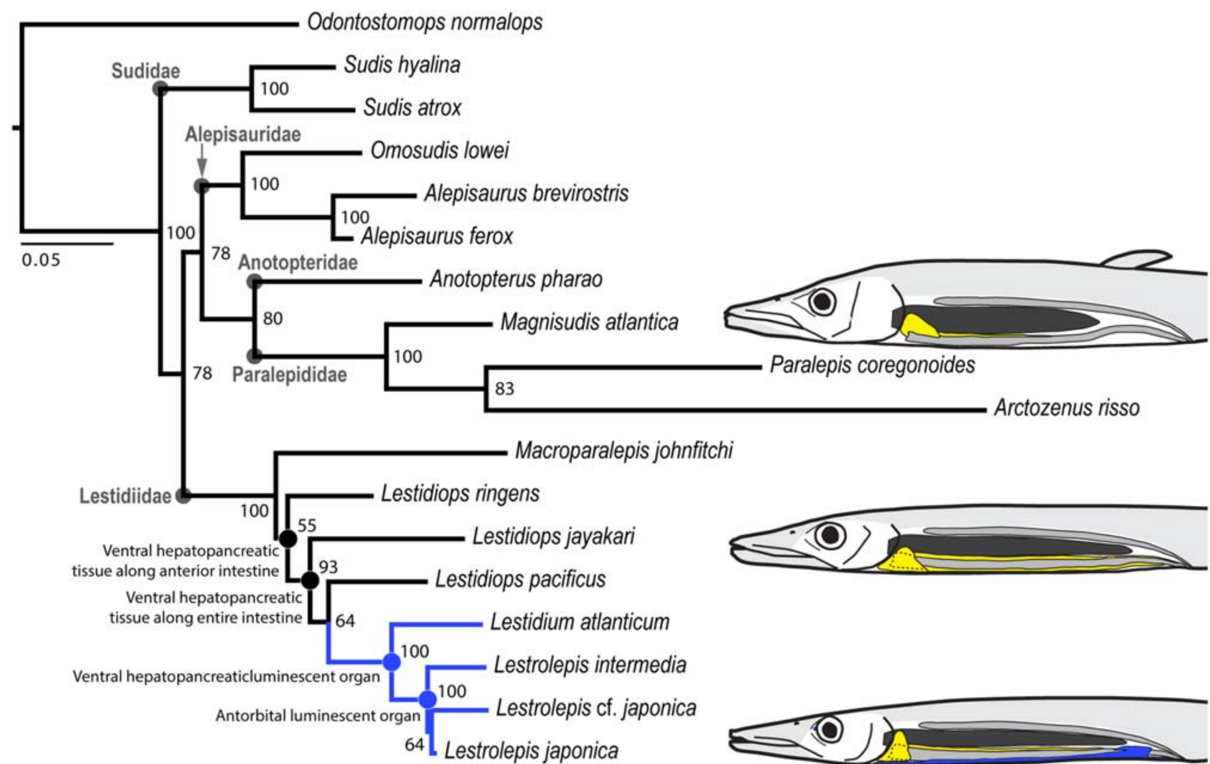

Fig. 4. Evolutionary relationships between representative alepisauroid fishes based on 8 sequenced gene fragments compared using maximum likelihood analysis. Blue lines in the phylogeny indicate the evolutionary events (apomorphies) of *Lestidium* and *Lestrolepis*. Numbers by nodes indicate results from 100 bootstrap replicates. Family names are indicated in grey at the nodes. (Ghedotti, Barton, Simons, & Davis, 2015).

Furthermore, it is apparent from information gleaned from gross dissection that the hepatopancreatic organ that became coopted into the bioluminescent organ began as an extended ventral tissue in *Lestidiops* spp., then becoming bioluminescent within the

Lestidium and *Lestrolepis* genera (Fig. 4). The evolution of an antorbital light organ in the genus *Lestrolepis* sets these species apart from *Lestidium*, which lacks such a light organ.

Discussion

Lestidium and *Lestrolepis* possess a ventral luminescent organ derived from hepatopancreatic tissue. It must be noted that this is the first instance of endogenous bioluminescence originating in hepatopancreatic tissue in any vertebrate. The luminescent quality of this organ is further supported by the cellular structures, as the distinct granular inclusions within the cells comprising the organ have previously been described as endogenously luminescent cells in other deep-sea fish species, such as *Coccorella* sp. (Herring, 1977). Furthermore, the histological data provided above allows me to clarify that the ventral "lens" described by Haneda (1964) is indeed smooth muscle derived from the peritoneum (such lenses are common in bioluminescent species for the sake of focusing and enhancing emitted light; Herring, 2002).

Lestrolepis possesses an antorbital light organ, which may serve light-matching purposes. As downwelling light enters the eye, the photophore adjusts to emit a similar intensity of light. By an unknown mechanism, the perception of light intensity in the eye is then relayed to the ventral luminescent organ, which then matches and emits the same intensity of light. This allows for accurate light matching and effective silhouette removal. The antorbital light organ is not observed in other Lestidiid fishes.

The phylogenetic data indicates that a ventral hepatopancreatic strand extending

posteriorly underneath the intestine evolved first in the common ancestor of *Lestidiops, Lestidium,* and *Lestrolepis*. Bioluminescence in this hepatopancreatic organ then evolved in the common ancestor of *Lestidium* and *Lestrolepis*. Finally, *Lestrolepis* gained the antorbital light organ, allowing for variation in the intensity of light emitted from the ventral luminescent organ. This may indicate that *Lestrolepis* has a greater ability to move up and down within the water column as compared to *Lestidium*.

Chapter 2 – Analysis of Communication Genes in Bioluminescent Bacterial Species Symbiotic with Deep Sea Luminescent Fish

Introduction

Marine fish bioluminesce either directly or indirectly: the first method is achieved by making endogenous proteins to carry out the light-producing reaction, and the latter is achieved by housing bioluminescent bacteria in a light organ. Many bioluminescent fish species house such symbiotic bioluminescent bacteria in extensions of their gastrointestinal tract, which allow the fishes to partake in predator avoidance and communication with potential mates (Dunlap & McFall-Ngai, 1987; Chakrabarty et al., 2012). My interests currently reside in genetic examination of these symbiotic bacteria, especially within the genus *Photobacterium*, which is present in many fishes, including the ponyfishes (Leiognathidae), sweepers (Pempheridae), and glow bellies (Acropomatidae; Kaeding et al., 2007; Haddock et al., 2010). Host species direct the establishment of pure cultures within their light organs by controlling microenvironments (e.g., specific gas and nutrient levels) in which only certain species and certain strains can survive (Dunlap & Kita-Tsukamoto, 2006). The availability of bacteria within the host's habitat largely determines which bacteria will live in the light organ as well (Ruby, 1996; Dunlap & Kita-Tsukamoto, 2006).

These groups of bacterial cells will only begin producing light once a sufficient

concentration of cells and signal molecules accumulates within the symbiotic colony, a phenomenon known as quorum sensing (QS). During QS, individual cells release signaling molecules for bioluminescence as well as other processes, and the desired action will occur once a threshold concentration of signaling molecules has been reached (Fuqua 1994; Shrout et al., 2011). QS controls processes including production of extracellular enzymes, biofilm formation, antibiotic synthesis, and bioluminescence (Dunlap & Kita-Tsukamoto, 2006; Roy et al., 2010; Galloway et al., 2011). Within Gram-negative bacteria, the group of bacteria to which the bioluminescent species belong, the primary QS molecules belong to the class of molecules known as *N*-Acyl homoserine lactones (AHLs), (Dunlap & Kita-Tsukamoto, 2006; Roy et al., 2010; LaSarre & Federle, 2013). The activity of AHLs, as they are implicated in bacterial pathogenicity, is an intriguing target for QS suppression and for understanding the foundations of bacterial communication. Thus, bioluminescent bacteria "turn on" their luminescence when the proper molecules are present.

Quorum sensing controls the expression of a group of genes called the *lux* operon, which contains the genes for light-emitting reactions. Essentially, all genes in an operon will be expressed (protein products produced) simultaneously as long as an initiating switch is triggered (e.g., by an AHL molecule). Most species exhibit increased luminosity with increased density of bacterial cells, indicating that the *lux* operon is directly controlled by highly concentrated AHLs (Rosson & Nealson, 1981). Indeed, the gene *luxR* codes for an AHL receptor and also acts as the controller of the entirety of *lux*

operon genes, demonstrating the relationship between QS and bioluminescence (Dunlap & Kita-Tsukamoto, 2006; Roy et al., 2010; Shrout et al., 2011; LaSarre & Federle, 2013).

However, it has been proposed that although multiple species control *lux* operon expression with QS AHLs, the evolution of these two processes occurred independently (Dunlap & Kita-Tsukamoto, 2006). Both *Photobacterium leiognathi* and *Photobacterium phosphoreum* indicate no density-dependent luminosity, and indeed they have been shown to constitutively express *lux* genes, such as those coding for luciferase, the enzyme that catalyzes the bioluminescent reaction (Katznelson & Ulitzur, 1977; Rosson & Nealson, 1981; Dunlap & Kita-Tsukamoto, 2006). Luminescence and quorum sensing have separate evolutionary origins and are not necessarily linked processes.

The reciprocal process of quorum sensing is quorum quenching (QQ). QQ is the mechanism by which QS signals of one species are destroyed by another species, thereby preventing other species from flourishing (Dong et al., 2001; Romero et al., 2011; Pereiera et al., 2012). By preventing transcription of QS-controlled genes, QQ confers a competitive advantage to the species employing it. In the context of symbiotic bioluminescent bacteria, QQ may allow for establishment solely of the bacteria using it within a fish light organ, as QS reportedly controls genes that give the bioluminescent bacterial species *Aliivibrio fischeri* the ability to colonize fish light organs (Lupp & Ruby, 2005). Therefore, targeting these QS molecules via QQ may inhibit the colonization of certain bacteria within a host species' light organ.

Quorum quenching thus may have evolved in bioluminescent bacteria, as it would allow for suppression of unwanted species within a light organ. Weiland-Bräuer et al.

(2014) have identified a QQ gene within an unidentified *Photobacterium* species that was grown from the surface of a species of seaweed. *Photobacterium leiognathi* is a bacterium that often resides within fish light organs, symbiotically producing light for the host species. I hypothesize that symbiotic strains of *P. leiognathi* will contain QQ genes such as those identified by Weiland-Bräuer et al. (2014), as these would allow *P. leiognathi* to suppress the activity and perhaps the colonization of other bacterial species within host light organs. Secondly, the generation of DNA sequence data will allow me to test the relationships among *Photobacterium* symbionts depicted in Wada et al. (2006). This analysis used questionable phylogenetic methods, making this an opportunity to test their hypothesis of substantial host specificity in *Photobacterium.*

Methods

Bacterial Strain Specimens

The specimens used in my analysis were one strain of *Aliivibrio fischeri* and one strain of *Photobacterium leiognathi*. I attempted to culture *P. leiognathi* from ponyfish specimens (*Leiognathus* sp.) purchased at a Vietnamese fish market in southwest Denver, CO. However, this was unsuccessful, likely because the fish had previously been frozen. Next, I tried to amplify various bacterial genes from these ponyfish specimens, including *Photobacterium Lux*A and 16S RNA, which yielded no substantial amplification of these genes. Additionally, I attempted to culture the non-symbiotic *P. phosphoreum* from the surface of these ponyfish specimens, as described by Ast and Dunlap (2005). This process, whereby I placed the specimen in a sterile tray, covered it with artificial

seawater, and allowed it to incubate at 6° C for six days, produced no luminous colonies of *Photobacterium*. Thus, I turned to purchasing a *P. leiognathi* culture from ATCC (American Type Culture Collection). I purchased the *P. leiognathi* ATCC 25587 (isolated by Boisvert et al., 1967), which is a strain isolated from an unidentified leiognathid fish (i.e., a ponyfish) light organ. *A. fischeri* was purchased from the company Carolina Biological.

All specimens were grown on low-salinity water (LSW) agarose plates. Per liter, LSW contains 10 g tryptone, 5 g yeast extract, 15 g agarose, 700 mL artificial seawater (Instant Ocean) and 300 mL deionized water. Both *P. leiognathi* and *A. fischeri* were subcultured from their original states (freeze dried and slant, respectively) in LSW broth, then plated after suspension. Cultures were grown at 25°C in darkness. In order to maintain viable colonies, subcultures were conducted every four days, and glycerol stocks were prepared for freezing –70°C. For glycerol stock preparation, LSW broth cultures were grown overnight. 1 mL of each culture was added to 0.25 mL 50% glycerol, vortexed, and stored in a –70°C freezer.

DNA Extraction

Single colonies from both *Photobacterium leiognathi* and *Aliivibrio fischeri* subcultures were picked and subcultured to establish monocultures. These monocultures served as the strains from which DNA was extracted. DNA extraction was conducted using a QIAGEN DNeasy Blood and Tissue DNA extraction kit. First, a large streak of cells was collected and suspended in 75 microliters (µL) LSW broth in an Epi tube using

a sterile loop from each of the prepared monocultures. After centrifugation, the pellet, which contained the bacterial cells, was isolated and suspended in 20 µL proteinase K (lyses the cells so DNA can be accessed) and 180 µL of ATL buffer (destroys nuclease activity and aids in cell lysis). The suspension was then incubated at 56°C for 6 hours. Next, 200 µL AL buffer (contains guanidine salts, which facilitate binding of DNA to the column and acts as a detergent to further break down cellular membranes) and 200 µL EtOH were added and vortexed. The solution was then pipetted onto the spin column provided (this will ultimately allow for the differential elution of cellular components – cell membrane, proteins, etc. – and DNA). After centrifugation, the flow-through was discarded, and the column was washed with 200 µL AW1 (contains guanidine to facilitate breakdown and passage of protein through the column). This step was repeated using AW2 (a tris-based ethanol wash that removes salts from the column). Lastly, 200 µL AE buffer (elution buffer that allows for passage of DNA through the column) was added and the column centrifuged. The eluted solution contained purified sample DNA, and this was stored at – 40°C.

Gene Amplification

DNA sequences *Photobacterium leiognathi Lux*A (abbreviated PLluxA), *Aliivibrio fischeri Lux*A (AFluxA), *Photobacterium* sp. quorum quenching protein No. 34a (QQ 34a, see Weiland-Bräuer et al., 2014), and 16S RNA (16S, a subunit of bacterial ribosomes, acts as control amplification sequence) for were amplified using standard polymerase chain reaction (PCR) protocol and the following primers. The primer

sequences are as follows: PLluxA-F (5’CATGATTTGGGCGAAAACCT-3’), PLluxA-R (5’-GAACCGTTTGCTTCAAAACC-3’, Wada et al., 2006); AFluxA-F (5’-GGTACCATGAAGTTTGGAAATATTTG-3’), AFluxA-R (5’-GGATCCTTTAGGTCCTTTTAAGAAAG-3’, Tehrani et al., 2012); QQ 34a-F (5’-GAATCGCTTCAATGATTCAGGCAGGTTAT-3’), QQ34a-R (5’-GAATTCTTAATTAAGATCCACCAC-3’, Weiland-Bräuer et al., 2014); 16S-F (5’-AGAGTTTGATCCTGGCTCAG-3’), 16S-R (5’-ACGGCTACCTTGTTACGACTT-3’, IDT ReadyMade™ Primers).

For each polymerase chain reaction, four amplifications were run for both DNA extracts. Using illustra PuReTaq Ready-To-Go PCR Beads (used as these produced better yields of amplification products than preparing master mixes in the laboratory), I added 5 μL of genomic DNA (*Photobacterium leiognathi* or *Aliivibrio fischeri*), 2.5 μL of each primer (forward and reverse for the gene of interest), and 15 μL sterile, deionized water. After mixing, the reactions were run using a thermocycler with the following parameters: 95°C for 1 min (denaturation), 49°C (primer annealing), and 72°C (extension), which was repeated 30 times and preceded by a 5 min primary denaturing stage and a 10 min final extension stage (same temperatures as cyclic denaturation and extension used). Each reaction was run in triplicate. The PCR products were then analyzed using gel electrophoresis. To the agarose gels (50 mL TBE with 0.875 g agarose) I added 5 μL of Bullseye DNA Safe Stain, which allowed better visualization of bands. Samples were mixed with 2 μL loading dye prior to loading. Each well was loaded with 20 μL sample, and 5 μL of Bullseye 100 bp ladder was loaded as well (ladder band base pair lengths:

1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100). Gels were run for 1 hour at 100 V and imaged on a UV light using an iPhone 5.

Gene Sequencing

Samples were sent out for Sanger sequencing at Functional Biosciences in Milwaukee, WI. In preparation for sequencing, each of the desired genes was amplified from genomic DNA using PCR. *Photobacterium leiognathi* was sequenced for *P. leiognathi Lux*A, QQ No. 34a, and 16S RNA, while *Aliivibro fischeri* was sequenced for *A. fischeri Lux*A, *P. leiognathi Lux*A, and 16S RNA. All sequencing reactions were run only with the forward primer and yielded clean data except QQ No. 34a.

Sequence Analysis

DNA sequence data was compared from 32 new and published bacterial gene sequences from two genera (*Aliivibrio* and *Photobacterium*) within the family Vibrionaceae (Wada et al., 2006). *Aliivibrio fischeri* functioned as the outgroup in the analysis. Two genes were used: 16S ribosomal RNA (1,481 bps) and the gene coding for LuxA protein (10,820 bps). Therefore, a total of 12,301 base pairs were used for each species. The genes were aligned (process by which multiple genes are aligned to assess sequence homology, which allows for elucidation of shared evolutionary origins between these genes and ultimately the organisms themselves) using the program MAFFT v7.2 (Multiple Alignment using Faster Fourier Transform, a multiple sequence alignment program for comparison of gene sequences), run using the program's default parameters.

A maximum-likelihood analysis was then conducted in order to estimate the most likely pattern of genetic evolution for each gene and each category of position within each gene. The models of molecular evolution were selected using jMODELTEST v.2.1 (Posada, 2008), which assigned the most probable model of evolution for each of the eight compared genes (done using Akaike information criteria, essentially a measure of the quality of the estimated models; HKY+G, GTR+G were the models of evolution used for the *Lux*A gene and 16S RNA gene, respectively). The program GARLI v2.01 (Genetic Algorithm for Rapid Likelihood Inference) was then used to generate the most likely phylogenetic tree based on the maximum likelihood data in the context of the genetic models of evolution (Zwickl, 2006). The most likely phylogenetic tree was then depicted and manipulated using FigTree v1.4.

Results

*Lux*A *Analysis*

Amplification data indicates that both the *P. leiognathi* and *A. fischeri* strains analyzed possess copies of *Lux*A (Fig. 5. B, F; 1200 and 1000 bp, respectively). The *P. leiognathi Lux*A primer set did yield an amplification product when combined with *A. fischeri* DNA, thus this was sequenced (Fig. 5. E; 750 bp).

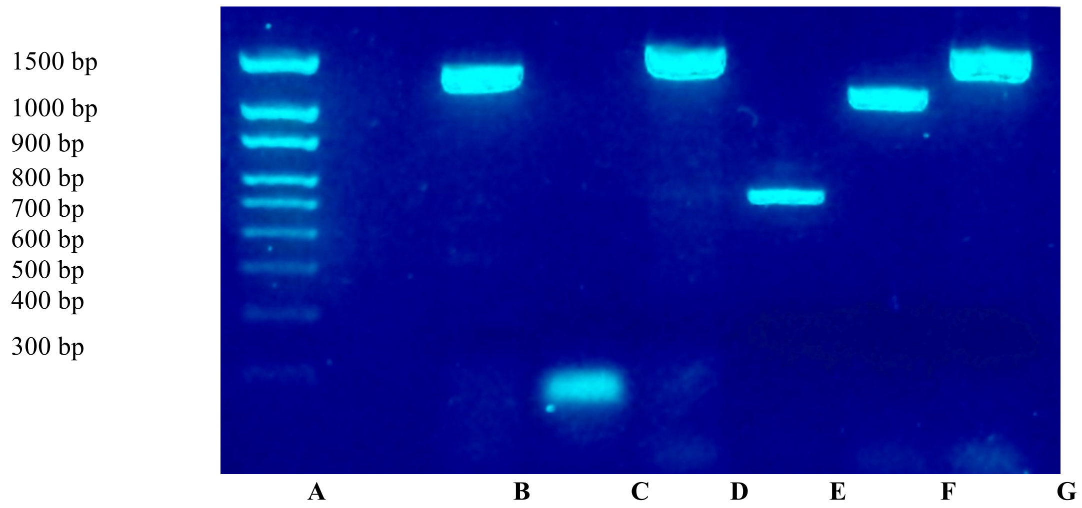

Fig. 5. PCR amplification products for *Photobacterium leiognathi* and *Aliivibrio fischeri* genes of interest. From left to right, lanes are as follows: (**A**) 100 bp ladder, (**B**) *P. leiognathi Lux*A, (**C**) *P. leiognathi* QQ No. 34a, (**D**) *P. leiognathi* 16S RNA, (**E**) *A. fischeri P. leiognathi Lux*A, (**F**) *A. fischeri Lux*A, and (**G**) *A. fischeri* 16S RNA.

Quorum Quenching Analysis

Upon gene amplification and sequencing, it was demonstrated that *P. leiognathi* ATCC 25587 does not possess the quorum quenching gene of interest, QQ No. 34a. While some gels did present very faint bands of the expected size (~600 bp), none demonstrated sufficient amplification (Fig. 5. C indicates a band of about 100 bp in size, likely a dimerization of primers). Furthermore, sequencing of crude PCR products amplified with the QQ primer set did not indicate any valid sequence for a quenching gene.

Phylogenetic Analysis

The strain of *P. leiognathi* examined in this study, ATCC 25587, is nested within a group of *P. leiognathi* strains known to be symbiotic with fishes from the family

Leiognathidae (Fig. 6.). Specifically, it resides within a clade of bacteria specific to the ponyfish species *Leiognathus rivulatus* and *Leiognathus nuchalis*, both from Japan, as well as *Leiognathus equulus* and *Leiognathus leuciscus*, both from the Phillippines. Furthermore, the *P. leiognathi* strains known to be symbiotic with *Siphamia versicolor*, a species found off the shores of Japan, form a monophyletic group. *P. leiognathi* strains symbiotic with Japanese *Acropoma japonicum* do not form a monophyletic group, and are thus found in clades containing *Cocorella* sp. strains gathered from the seafloor.

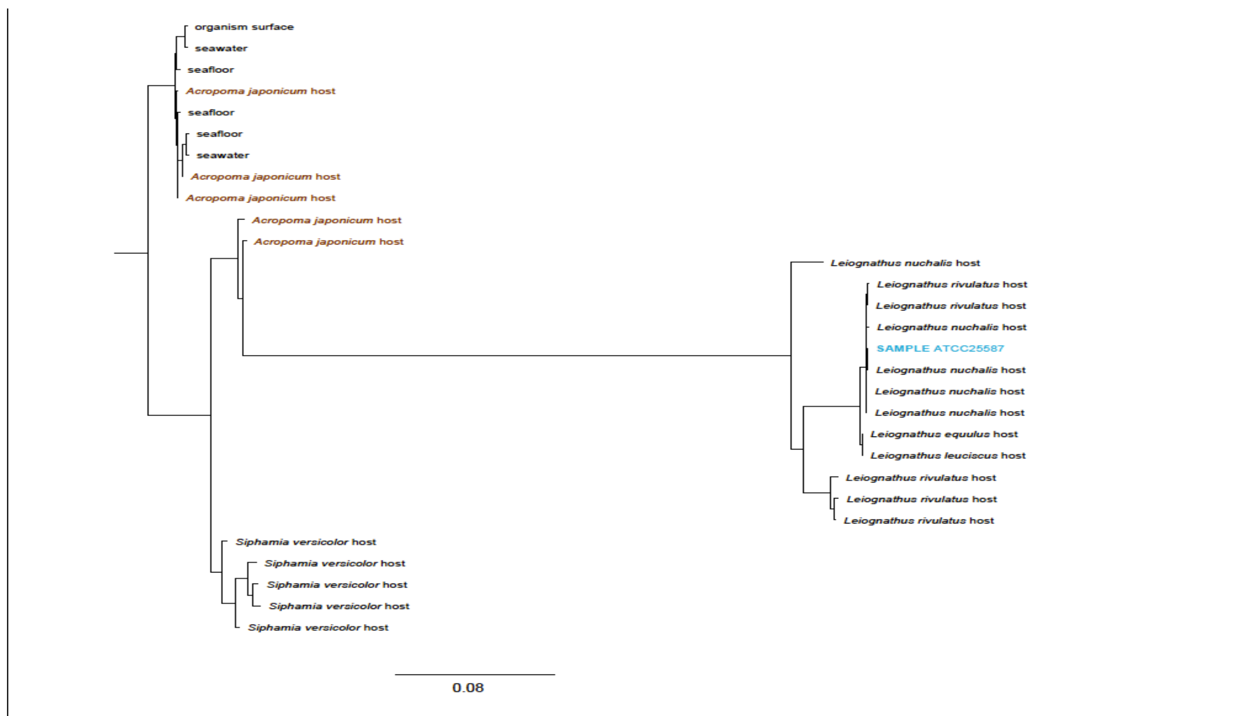

Fig. 6. Evolutionary relationships between bioluminescent marine bacteria based on sequence data of 2 sequenced gene fragments and sequence data from Wada et al. (2006) compared using maximum likelihood analyses. Each taxa represents a *Photobacterium* strain indicated by its host fish species.

Discussion

The initial hypothesis of this study, that QQ genes would be present in in *P. leiognathi*, must be rejected based on the sequence data. Due to time and financial

restrictions, only one species of symbiotic *P. leiognathi* was analyzed in this study, while many more strains exist and should be examined in the future to definitively determine the presence or absence of the tested QQ gene.

Phylogenetic analysis allowed us to verify the *P. leiognathi* strain obtained from the culture collection and examined in this study. Thus, we are able to support that *P. leiognathi* ATCC 25587 is likely a symbiotic strain obtained from a leiognathid fish. This is important in that it allows this strain of *Photobacterium* to be properly aligned with other symbiotic bacteria where the evolution of bacterial strains can be shown to evolve in a host-dependent manner. While QQ was not shown to be present in the strains examined herein, the formation of clades with high specificity for a particular species or genus of fish indicates that similar strains of the *Photobacterium* genus may possess characteristics that contribute to this specificity. If QQ does indeed allow for the inhibition of growth of competing strains of bacteria, this or a similar mechanism may be found within other strains of *P. leiognathi* upon further research.

Also, the phylogeny created in this study supports the specificity between *Photobacterium* strains and host species for *Siphamia* and leiognathid symbionts as Wada et al. (2006) had indicated. Acropomatid symbionts are separated with some strains in groups with *en vivo Photobacterium* species or leiognathid symbionts. Lastly, the group of leiognathid symbionts indicates large sequence divergence due to the long branch for their clade in the above phylogeny, supporting greater specialization within this clade of *Photobacterium* symbionts.

Chapter 3 – Experiential Learning: The History, Importance, and Practicality of Learning by Doing

In conducting all of the above research, research about which I am very passionate and driven, I did have one question in mind: what is the purpose? What do histology, morphology, anatomy, and phylogenies matter? As a biologist, these questions came to me as sacrilege, but they came to me nonetheless as I try to understand my place in this world and to what our actions ultimately amount. Looking back at my four years of education at Regis, I realize I have arrived at a much different place than I began as a freshman. My passion for certain things, such as biology, has not faded, yet I feel as though the scope of my view has grown. I understand this increased comprehension comes along with maturity, yet I do not enjoy that I know *more*. I enjoy that I am beginning to see beyond myself and take in the full importance of other people and places. I have begun to relish in my experiences in this world, as this is how I relate to all. My career at Regis has been a microcosm of this realization, and the research experiences I have detailed above are just a sample of the many ways in which I have started to look beyond my thoughts and desires to the wide empirical world around me.

Learning by experience, then, has been an integral aspect of my college education so far. I often find myself immersed in books during my learning, and of course, a complete education requires that one study the canon of work completed in a field in order to understand the accomplishments and advances made by previous academics.

Furthermore, the student should seek a full understanding of the basis of any concept, for without this knowledge progression is impossible. However, in order for the student to make any substance of lectures and readings, one must engage the world at large. What happens once the student steps out of the lecture hall? Armed with knowledge, the student makes nothing of these efforts at understanding if he or she does not deliberately *go* into the world and actively apply what has been bestowed upon him or her.

My fascination with understanding just what our experience in the world amounts to began my sophomore year of high school, during which I read Henry David Thoreau's *Walden*, where he states in reference to university students, "I mean that they should not *play* life, or *study* it merely, while the community supports them at this expensive game, but earnestly *live* it from beginning to end. How could youths better learn to live than by at once trying the experiment of living?" (Thoreau, 1854, p. 45). I found Thoreau's assertion entirely fascinating. I began to consider his appellation for students to "earnestly *live* it," to make good on their education. We receive a great deal of book knowledge, of information that anybody can store in his or her head. Even the advice or stories I hear from other people about their lives, outside of the academic setting, falls into this category. We constantly take in information; the input is incessant when you think about it. But what Thoreau wanted to convey is that this information must be acted upon. Life is not lived by thinking about living, but one must attempt the experiment of living immediately.

Experiential Learning Defined and Applied

As my education progressed, I began to think about the importance of experiential learning. This seemed to embody this existential mindset of Thoreau's in a way that made the education I was given seem worthwhile. Simply put, experiential learning is "…learning by doing" (Day, 2012). There are numerous models of experiential learning that will be dealt with later, but the basic idea is that experiential learning is not "…passive learning in which students listen to lectures, read books, or watch video documentaries, and are then asked to repeat what they have heard, read or seen", but rather it is "Active learning…in which students learn from field examples, data analyses, case studies or problems…" (Day, 2012, p. 5). The intriguing aspect of experiential learning is the action it requires of the student. This "active learning" requires action rather than passivity, which resonates with how I believe learning can become reinforced in one's mind. While lectures and readings may be seen as passive, I believe that the entire learning process can be made active. As long as the student remains engaged in the subject matter, which may mean debating or conversing with a peer on the topic, then the student is engaging in the active learning to which Day refers.

Furthering the philosophical understanding of experiential learning, John Newman in *The Idea of a University* offers another kinetic definition of learning. "It is not the mere addition to our knowledge that is illumination," Newman posits, "…But the locomotion, the movement onwards, of that mental centre, to which both what we know, and what we are learning, the accumulating mass of our acquirements, gravitates" (Newman, 1996, p. 98). Newman calls upon two very important concepts involved in the

human act of learning. First, he reemphasizes the commonality in both Thoreau's and Day's observations of learning that it ought to involve a sort of motion, or "movement onward" of one's understanding. When stagnation or passivity becomes the *status quo*, "mere addition" to one's knowledge is the only result. This is no good, for the student does not reach any deeper understanding, and the experience remains superficial such that the learning will not culminate into anything substantial. Secondly, Newman begins to define precisely that which the inquisitive being is working to alter or improve in the first place: "that mental centre." In the process of learning, which may even be broadly understood as the process of life, the human being strives to adapt his or her consciousness, ever refining and ever clarifying that grasp of oneself and the world. It is not "our knowledge" that we seek to improve through edification, for this is only one aspect of this mental centre. It is my purpose in this chapter to comment on whether broadening one's outlook on the world can allow for a comprehension that offers us a satisfaction with our mental centre, whether that be in the school setting, community setting, or simply in interacting with other people.

The majority of the discourse on experiential learning has occurred within the field of behavioral psychology, a field largely concerned with explaining how we learn. The main players who initially explained experiential learning are Kurt Lewin, John Dewey, Jean Piaget, and David Kolb. Kolb offers a synthesis of the work done by Lewin, Dewey, and Piaget, claiming experiential learning is, "…A holistic, integrative perspective on learning that combines experience, perception, cognition, and behavior" (Kolb, 1984, p. 21). Kolb's emphasis on the holistic aspects of a learning process founded

on experience and reflection on that experience embodies experiential learning. Kolb further develops the holistic nature of experiential processes in his claim that learning "…involves the integrated functioning of the total organisms" (Kolb, 1984, p. 16). The entire human organism engages in the learning process, for learning takes place in the context of one's environment. In biology, students are taught that evolution of species occurs when species interact with their environment. In the same way, Kolb argues that learning is an adaptive process that must engage the entire human organism. Examination of the three learning models provided by Lewin, Dewey, and Piaget will further inform this stance.

Lewin's model focuses on feedback mechanisms in learning that allow for advancements in understanding. Lewin's model stresses the balance between observation and action, and these two feed into each other, as can be seen in Fig. 7. In the learning process as defined by Lewin, a student is subjected to a concrete experience, which is followed by observations and reflections

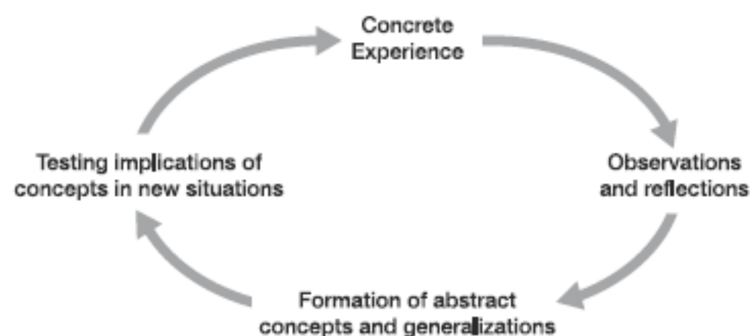

Fig. 7. The Lewinian model of experiential learning (figure from Kolb, 1984, p. 21, Fig. 2.1).

on that experience. Next, the student will take the observations and reflections to formulate abstract concepts about the original concrete experience. These hypotheses lead to testing these concepts in new situations and contexts, ultimately yielding new concrete experiences. The cycle then repeats, and the student begins to understand the relations of a set of phenomena within a given set of contexts. Importantly, Lewin places emphasis on "…Here and now concrete experiences" that "…Yield subjective personal meaning" (Kolb, 1984, p. 21). Lewin strives to situate the student, and the student's meaning, in the learning process. Rote memorization and superficial understanding of topics do not interest Lewin. Rather, his model indicates that the student's thinking is directly linked to the cycle of events connected to the observations and experiences of the student's life. In other words, a person does not merely possess thoughts distinct from the phenomena surrounding him or her, but rather those thoughts are a part of and a contributing factor to the comprehension of those phenomena.

Dewey provides a model that stresses the transformative effects of experience on the student's purposefulness. Akin to Lewin, Dewey's model demonstrates "…How learning transforms the impulses, feelings, and desire of concrete experiences into higher-order purposeful action" (Kolb, 1984, p. 22). Thus, the student begins with impulses, curiosities, and experiences in observing phenomena around him or her. These are all great for an inquisitive novice, but eventually he or she wants to bring learning to fruition, to transform that raw material into some "purposeful action." Looking back to Newman and his discussion of the "locomotion…of that mental centre" in order to truly learn, one immediately notices the parallels with Dewey's model of learning. As seen in

the diagram of Dewey's model (Fig. 8), the learning process is one of forward movement, but still retaining the cyclic analysis characteristic of the Lewinian model. Advancement in this cyclic-progressive mode is founded on deriving a purpose from original impulses founded in nascent observations and curiosity.

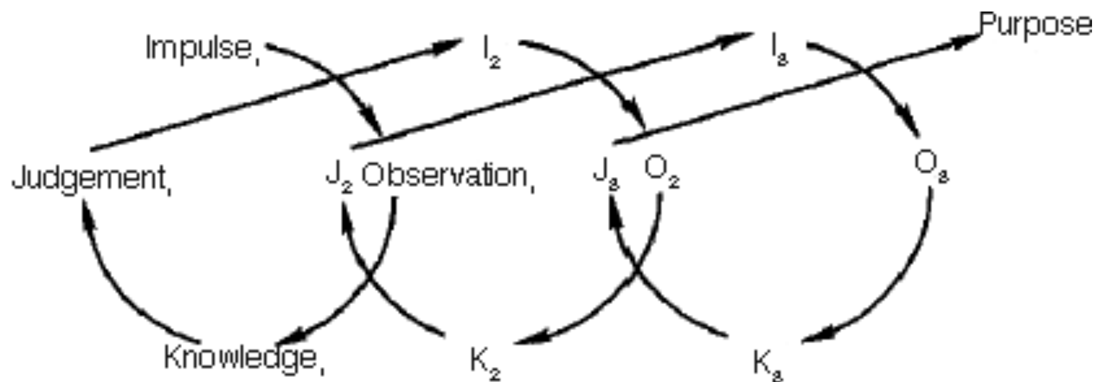

Fig. 8. Dewey's model of experiential learning (Kolb, 1984, p. 23, Fig. 2.2.).

Finally, the learning model posited by Piaget emphasizes the adaptation of the student to synthesize ideas. The four processes of Piaget's model are experience and concept, reflection and action (Kolb, 1984). His model resembles the Lewinian model, as experiences lead to concepts (derived via reflection) that lead to action, and eventually to the broader goal of adult thought. Once again we see the acquisition of purposefulness or meaning as the goal of the entire learning endeavor. This has been an important driving factor in my interest in experiential learning, as I always felt disturbed by the emphasis on grades and tangible achievements in the education system I experienced. I believe that in school, and in life, the data, impulses, and observations one has should not be

primarily reworked to yield some material end goal, but rather to give the subject a greater meaning and a broadened perspective.

Therefore, learning ought to be envisioned as a process rather than an outcome. Learning is a continual process that never ceases. Learning, rather than existing as a fixed event in time, is a process that constantly adapts. One can therefore view ideas as Kolb does, as mutable events that are continually reformed in one's daily life. The dialectic tension between experience and concepts, between the concrete and abstract, is that locomotive force of a mental centre. The experiential model presents learning as a process of tension, of trial and error that ultimately leads to a progression for the subject, and this progression should provide the student, at the very least, a meaningful purpose for engagements with the world.

Experiential Learning Applied

In order to give the Lewinian model a concrete context, I look back on my own research and learning in the laboratory. My interest and concern with experiential learning was very much initiated with my own engagements with variable learning process. The Regis Biology Department offered me multiple opportunities to get involved with exciting research projects in which I knew I had to become involved. While I learned a wealth of information in my biology courses and the laboratories helped place this knowledge into a context of greater understanding, I longed to find that "freedom and unity of… researching" to which Simões et al. refer (2015, p. 131). I aimed to put my

hands on new and tangible research projects and to make observations that had not before been made.

I thus began conducting research under M. Ghedotti's guidance. The study we conducted is previously outlined in Chapter 1, a study concerned with elucidating the structure, anatomy, and evolution of bioluminescent organs of the naked barracudinas. Needless to say, this was not a topic I had been studying my first two years of college. What I had learned about was cell structure, basic anatomy, basic development, staining, and evolution. All of these principles that I had learned in the classroom finally had a space to grow, a place for me to enact them and see how they operated in the world of science. Application of these ideas allowed for them to coalesce in my understanding.

I saw how these fish that live in a region of the ocean where downwelling light casts the shadows of their bodies to predators below have adapted literal lights on the bottom of their bodies in order to counteract these shadows. What a case of adaptation! Furthermore, I came to see how these fish had evolved such light structures, as proposed by the phylogeny in Figure 9. In this phylogeny produced by M. Davis and combined with the data collected by M. Ghedotti and myself, the process of evolution is readily apparent. Examining the family Lestidiidae, the uniquely derived characteristics that are present is first the development of ventral hepatopancreatic tissue along the anterior intestine (toward the head), then along the entire intestine, which was finally coopted to become bioluminescent tissue in *Lestidium* sp. and *Lestrolepis* spp. I give this example not to focus on the intricacies of the evolution of this group of fish, but rather to focus on the applications of evolution it represents. Indeed, this was experiential learning in action.

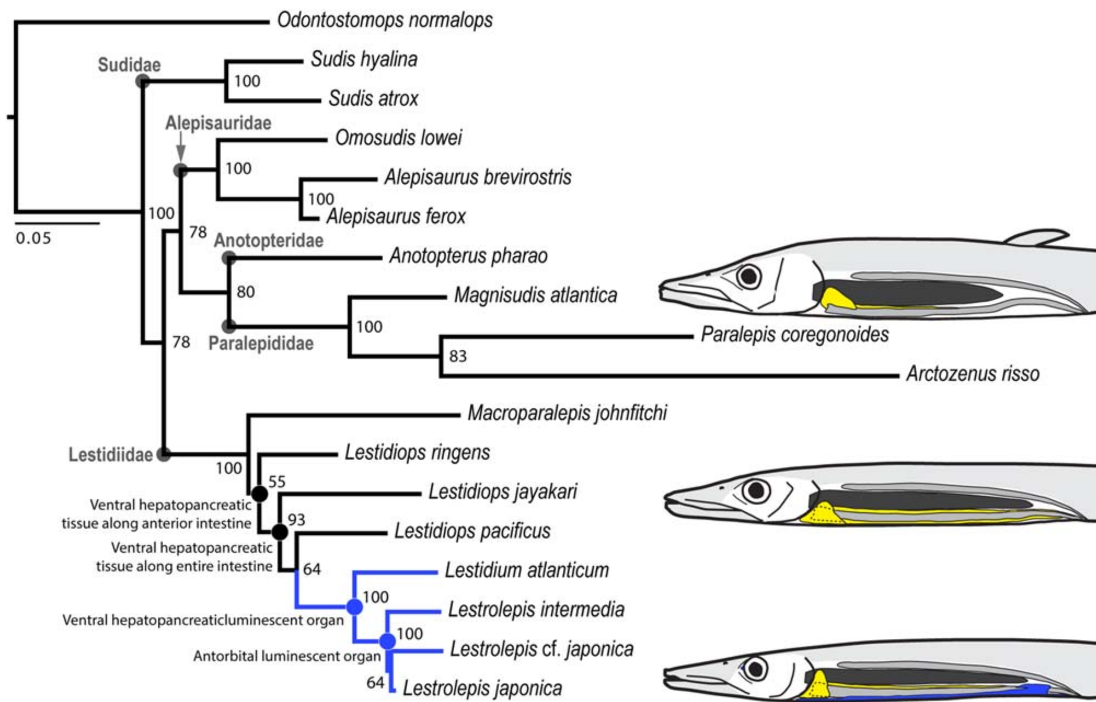

Fig. 9. Phylogeny of Lestidiid fishes and representative alepisauroid fishes. (Ghedotti, Barton, Simons, & Davis, 2015).

The next step in my experiential learning comes from my progression onto the research that is outlined in Chapter 2. First, however, I must return to the research I conducted with M. Ghedotti. Our original hypothesis with the naked barracudinas was that they were coopting gut diverticula, or elongated sacs extending off the beginning of the digestive tract and continuing along the length of the ventral side of the fish. Such diverticula formations have been observed in fish of the genus *Coccorella* (Wassersug & Johnson, 1976). However, this hypothesis had to be revisited once the histology of the Lestidiid fishes was thoroughly examined (see Chapter 1). Nevertheless, I became quite intrigued with those fish that do harbor luminescent bacteria in their gut diverticula, such

as the ponyfish. I began to wonder how colonies establish in these fish, as they are often pure colonies comprised of one species of bacteria.

With this curiosity derived from my first-hand observations of phenomena, I looked back at the literature. My reflection on the communication among bacteria led me to discover the processes of quorum sensing and quorum quenching (see Introduction of Chapter 2). In essence, I had moved from the "concrete experience" of the Lewinian model toward the "observation and reflection phase" (see Fig. 7). If only one species was forming a colony in each fish, such as in ponyfishes, did that mean some tactic was being used to prevent other species from establishing colonies? Were the quorum sensing processes that allow bioluminescent bacteria to luminesce as a cohesive unit being inhibited by other species attempting to do the same, in an effort at winning "colonization rights" in marine-fish light organs? These questions filled my head, and I was led to hypothesize that perhaps quorum quenching, a process by which bacteria inhibit the molecular signaling between competing bacterial species (e.g., signaling for light production), was occurring in the bacteria commonly colonizing fish light organs. This represented Lewin's "formation of abstract concepts and generalizations" stage. Finally, I began to research this subject, finding that perhaps these bacteria do not possess quorum quenching genes (see Results in Chapter 2). This "testing of concepts" phase is the final step in Lewin's cycle, and in the broader experiential learning cycle. Lewin's model of learning closely parallels the scientific process, which essentially consists of the observation-hypothesis-experimentation cycle I worked through in the laboratory.

Experiential Learning Provides the Basis for University Research

Returning to my original questions, concerning why I conducted undergraduate research for three years, I began to wonder where the concept of research in the university began. The conception of the research university as it is known today began with the 19th century German universities, which placed great importance on experiential learning. While German professors of the 1800s did not understand experiential learning as has been described above, they did understand the importance of observation, experimentation, and empiricism that led to the progress of the sciences seen at the turn of the 19th century and into contemporary times. Many of the foundations for incorporating research into learning institutions propel my own conception of experiential learning, and they deserve attention.

The idea that research should be conducted in the university setting stems from the 19th century German concept of *wissenschaft,* or "pure science." *Wissenschaft* was understood in universities as science for the sake of science, not for the sake of a professorship, the state, or a company (Nyhart, 1995). In his argument stating 19th century American medical students frequently traveled to Germany seeking further educations in the universities there, Bonner recognizes that these students met in "…The disinterested pursuit of truth" (Bonner, 1987, p. 2). The ethos of *wissenschaft* achieved a scientific process that focused on the education of the student, if not merely for the sake of piquing his or her curiosity. Indeed, both Bonner and Nyhart in their discussions of the 19th century German research universities emphasize the disinterested and laboratory-based approaches to teaching the life sciences, which effectively awoke the imagination

of the student (Nyhart, 1995). Or, as Bonner states, these approaches to learning initiated an "…Exciting sense of studying on the frontier of … discovery" due to the complete devotion of the laboratory approach to the spirit of science (Bonner, 1987, p. 16). The spirit embodied by the German style is a truly scientific one, for it is founded in constant questioning and pursuit of empirical knowledge that is centered within the professor- or student-scientist. It is at the interface between the individual and natural phenomena that science progresses, thus allowing for the further maturation of the student's understanding.

The German approach (emphasis on laboratory work, empiricism, etc.) arguably arose with the transition from traditional academies toward technical institutes, as the focus shifted from mere transmission of knowledge toward the holistic and personal advancement of knowledge (Clark, 2008, p. 437). In essence, this transformation is what is known as the move from a traditional university (the 'academy') toward the Humboldtian model (the 'research university'), the latter emphasizing "…the principles of freedom and unity of teaching and researching" (Simões et al., 2015, p. 131). This marks a move toward a more holistic approach in education, creating an academic environment focused not only on transmitting knowledge, but also on research (progression of knowledge) and the cultivation of a moral self with a core set of values (there exists a parallel between this ideology and the Jesuit teaching of *cura personalis*). An idealistic revamping of the university was under way at this institutional transition point

More specifically, this change can be traced to the creation of the Humboldtian model of the university. The Humboldtian model, named after Wilhelm von Humboldt, stressed the concepts of *wissenschaft*, academic freedom, reason, and logic. With the construction of the University of Berlin in 1810, the first university styled after the Humboldtian model, higher education took a turn away from the dogmatic academies toward a learning institution where empiricism, questioning, and academic freedom were priorities (Anderson, 2004). While the instructor's role remained an authoritative one, the Humboldtian university focused on unity between teaching and research (Anderson, 2004). The hierarchical system of the university had been abandoned to an extent, replaced by a format where instructors became highly involved in research that both informed what they taught their students, and provided an opportunity for the students to practically implement their learning. Students were taught a disinterested brand of science that held no teleological high ground. Any and every idea was subjected to empirical data, logical reasoning, and questioning by professors and students alike.

Additionally, the Humboldtian model puts a large emphasis on the idea of *bildung*, or "education contrasted with training" (Simões et al., 2015, p. 131). That is, *bildung* represents education beyond simple conveyance of knowledge, instead seeking to transmit moral and cultural knowledge (Simões et al., 2015). *Bildung* was to be taught with a dynamic education process, which the German universities sought to achieve by combining lecture material with research and learning laboratory experiences (especially in the natural sciences). Indeed, Nyhart expands on this definition by stating *bildung* is the "development of self to its highest potential" (Nyhart, 1995, p. 14). Nyhart's

understanding of learning development as a maturation of the self's "highest potential" is not far removed from Newman's conception of illumination being a movement toward a "mental centre," for both of these loci represent an anchoring point to which our efforts in education are centered. In cultivating our "mental centres" or "highest potentials" we strive toward the goal of education: a better understanding of the world around us by examining and contemplating the phenomena that fill it. The "mental centre" of one's being represents that potential to understand and sincerely engage with that which surrounds him or her.

The process of transferring knowledge in an experiential manner has its foundations in the German ideals of education, namely *wissenschaft*, *bildung*, and the Humboldtian model. In pushing for freedom in teaching and researching, students began to experience the transmission of knowledge at a practical level. The care for the entirety of the student's learning process hearkens back to Kolb's assertion that experiential learning "…involves the integrated functioning of the total organism" (Kolb, 1984). The aspects the behavioral psychologists focus on certainly are similar to the facets of learning the Humboldtian model strove to emphasize. Both seek a sort of attention to the entirety of a person, what I can best describe using the Jesuit conception of *cura personalis*, or care for the whole person. The German universities focus on teaching the students the canon of knowledge relevant to the field of study with which the student engages, but the inclusion of research adds a counterpoint from which the student can test these ideas and survey them further in the world at large. Experiential learning is simply a microcosm of this very same ideal. The student experiences and perceives, then engages

cognitively with the data, and initiates behavior based on the prior cyclical steps. Essentially, both concepts of learning, the German research university model and the experiential model, are one in the same: they place emphasis on the propulsion of intellect through the means of fortifying *a priori* knowledge with empirically gathered *a posteriori* knowledge.

Throughout this discussion, the concept of what truly constitutes learning has remained at the center. The foundations of learning are indeed the foundations of our civilization and our daily lives. The human race continually learns, through every experience of each day, even if it does not leave a lasting impact. As a scientist, I have always seen the importance of continually questioning phenomena I observe in the living organisms, inanimate structures, and communities of people that surround me. In a sense, the shift toward the Humboldtian university and the creation of the research university parallels this line of thinking quite closely. The shift toward the Humboldtian research university solidified the status of professors and students as a unified group engaged in the common search for truth (Anderson, 2010). The search for truth will never yield absolute truth, and this is the source of my own curiosity. I see the quest for understanding as a continually evolving one that resembles the evolution of species. There is no correct course of evolution or final teleological goal toward which evolving organisms tend. Rather, the evolution of my learning, as with organisms, is often influenced by chance and outside factors that the subject cannot control. In learning and living, these factors are experiences. There exists no map to find the best ones; there is only a fluid journey without a known destination. Forever there will exist a search, during

which the student, the professor, the person learns, learns a new way to behold the ebb and flow of existence. The promise of an answer does not drive one to research; rather, the certainty of more questions to be asked will forever pique the student's and the scholar's curiosity.

Conclusion

As I look back on my journey through Regis and reflect on the progression of my learning and maturation, two important Jesuit principles strike me as crucial elements to my development. The first is *cura personalis*, or care for the whole person. Care for the whole person entails spiritual, educational, and moral development, and I believe my emphasis on experience coincides well with *cura personalis*. By taking hold of each opportunity that presents itself, I am able to explore the world of science, the world of thoughts, and most importantly the world of the people around me. In communicating and learning from others, I am able to formulate my own ideas and reevaluate them constantly. In fact, I am nothing without the people with whom I form relationships, as they give me a foundation in this world upon which I may grow. In all, I am able to care for my whole person and become a well-rounded individual when I engage with the experiences of which I have the opportunity to be a part.

Secondly, *magis* is a very important Jesuit value that means much to me and informs my attempts to become a better student and a better person. *Magis* is difficult to define, but it directly translates as "more" from Latin. The Jesuits, however, use it to discuss for what we live. Do we live for ourselves, for small, selfish reasons, or do we live for something more? In the final Honors seminar, *Magis and the Search for Meaning*, my classmates and I contemplated what we live for and how we do that in great

depth. While my answers to these questions are not definitive and continually evolve, one important takeaway for me is that we can learn about our answers to these questions through the experience of life. Some prominent figures covered in the seminar were Father Gregory Boyle, who learned his meaning through working with gang members in Los Angeles, Viktor Frankl who found meaning while suffering through the concentration camps of Nazi Germany, and Andre Dubus who meditated extensively on life's purpose after becoming wheelchair-bound midway through his life. More generally, these people learned what the meaning of life was to them during these events that challenged them wholly to consider what it is they live for, or what the *magis* means in their lives. Therefore, it is the challenging of oneself to face adversity or enter uncomfortable situations that I find to be very important in my life. I am only a 22-year-old college student who does not know much, but I am excited with the prospect of encountering new experiences that will teach me about the complex world of people, places, and phenomena around me.

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