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Characterization of the Bioluminescent Symbionts from Ceratioids Collected in the Gulf of Mexico

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HALMOS COLLEGE OF NATURAL SCIENCES AND OCEANOGRAPHY

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ABSTRACT

Anglerfishes are easily one of the most popular deep-sea creatures due to their menacing appearance, extreme sexual dimorphism, parasitic mating approach, and eye catching bioluminescent lure. Unlike most bioluminescent fishes, which intrinsically generate light, female anglerfishes belonging to nine of the 11 families within the suborder Ceratioidei (deep-sea anglerfishes) have developed a symbiotic relationship with bioluminescent bacteria that are housed within the light organs. Previous molecular work had identified symbionts from two anglerfish species as novel and possibly unculturable taxa (Haygood *et al.*, 1992), but nothing more has been revealed about the bioluminecent symbionts of ceratioids. As part of the Gulf of Mexico Research Initiative-funded DEEPEND project (Deependconsortium.org), the objective of this study is to characterize the escal microbiome of deep-sea anglerfishes and identify potential-symbiont taxa.

A total of 36 anglerfish specimens were collected on DEEPEND cruises DP01 through DP04. These specimens consist of adult and larval individuals belonging to six of the families with the suborder Ceratioidei: Ceratiidae (n=22), Oneirodidae (n=7), Linophrynidae (n=3), Melanocetidae (n=2), Centrophrynidae (n=1), Melanocetidae (n=2), Gigantactinidae (n=1). DNA was extracted from esca, skin, fin, gill, gut, and caruncle tissues, as well as seawater. High-throughput sequencing of the 16S rRNA hypervariable V4 region was carried out using the Illumina MiSeq.

Sequencing revealed five potential bioluminescent-symbiont taxa (OTU IDs: 9129, 9131, 160210, 523223, and 939811), which had the greatest relative abundance (25.2% - 98.7%) within 12 of 21 adult specimens. These taxa belong to the family Vibrionaceae and were found at greater than 10% relative abundance in the escal samples of adult anglerfishes belonging to the Ceratiidae and Melanocetidae families, but they were not found in high abundance in larval individuals of the same families. Sequencing of larval samples revealed five potential bioluminescent-symbiont taxa (OTU IDs: 136178, 176420, 523223, 837366, 939811) which were of greatest relative abundance (8.1%-67.1%) within nine of 13 specimens. Also members of the family Vibrionaceae, these taxa were found in high abundance in larval anglerfishes belonging to the Oneirodidae, Linophrynidae, Gigantactinidae, and Ceratiidae families. This study is the first to to examine the bioluminescent symbionts from seven different ceratioid families.

Keywords: symbiosis, bioluminescence, Ceratioidei, microbiome, 16S

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INTRODUCTION

The Deep Sea

The deep pelagic is by far the largest ecosystem on the planet, accounting for over a billion km^3 (Costello *et al.,* 2010). The deep-pelagic zone is traditionally described as the offshore region of the water column between the ocean's sunlit surface waters and the sea floor. This region is often divided into zones based on depth. The surface waters, which lie above the deep-pelagic zone, are referred to as the epipelagic zone. This area constitutes the best-lit layer of the ocean stretching from the surface to a depth of 200 m. Below the epipelagic lies the deep-pelagic environment, which can be divided into the mesopelagic and bathypelagic zones. The mesopelagic zone stretches from 200 to 1000 m and is often referred to as the twilight zone because very little light penetrates to these depths. Even deeper, at greater than 1000 m, lies the bathypelagic zone, where the only visible light is that produced by bioluminescent organisms.

Despite their grand size, the meso-, bathy-, and abyssopelagic zones remain chronically underexplored due to the many challenges involved in studying this vast environment (Webb *et al.*, 2010). Although great strides have been made over the last half a century to reveal that the deep-pelagic environment is not the desert it was once believed to be (Grassle, 1989; ANGEL, 1993; Sutton, 2013; Irigoien *et al.*, 2014), our understanding of the life cycles and interactions between these unique organisms and their environment remains limited (Sutton *et al.*, 2017). Despite this the deep pelagial is not devoid of human impact. As of recent, the largest known threat to the deep-pelagic ecosystem in the Gulf of Mexico (GoM) was the *Deepwater Horizon* oil spill. As a result of the spill, a massive plume of oil was observed at a depth of approximately 1100 m (Camilli *et al.*, 2010). With deep-sea drilling and mining projected to continue if not increase (Thurber *et al.*, 2014), it is unlikely that the *Deepwater Horizon* blowout will be the last anthropogenic perturbation seen in the deep-pelagial.

Unfortunately, with a limited understanding of the ecology of the deep sea, it is difficult to extrapolate how such occurrences will not only directly impact the taxa within the region but how it may indirectly impact larger scale biological and physical cycles. Therefore it has become even more important that we continue to investigate not only the

organisms that call these dark waters home, but also gain a greater understanding of how they interact with and impact life around them.

Bioluminescence and Symbiosis

Often bioluminescence is the only form of light found at the deeper depths of the ocean. Bioluminescence is the production of light by a living organism, and it has been observed across roughly 700 genera within 17 different phyla. Of these, nearly 80% inhabit the oceans (Herring, 1987; Widder, 2010).

Bioluminescent light is generated via a chemical reaction that involves the oxidation of a light-emitting substrate, generically called a luciferin, by a catalyzing enzyme, luciferase (Hastings, 1996). Just as there is diversity in morphology and function, there is also variation in the molecular structure of these compounds across taxa. Of these two chemical components, luciferins are more conserved with four types accounting for most observed bioluminescence: bacterial luciferin, dinoflagellate luciferin, coelenterazine, and ostracod luciferin. On the other hand, identical luciferases are typically not shared across species (Haddock *et al.*, 2010). In some cases, organisms acquire luciferins from the external environment via their diet or symbiont acquisition, and this has been proposed as an explanation for the noted conservation of luciferin across unrelated organisms (Haddock *et al.*, 2010; Widder, 2010)

It has been estimated that bioluminescence has evolved independently at least 27 times within fishes (Davis *et al.*, 2016), which has given rise to a vast diversity in light organ morphology and function (Herring *et al.*, 2002; Shimomura, 2006). Fishes in particular demonstrate a vast assortment of photophore morphology ranging from simple groupings of luminescent cells to large, optically complex organs containing lenses, filters, and reflectors (Herring, 2000).

Along with this great diversity in morphology also comes noteworthy variation in function. Bioluminescence is thought to provide defense through counterillumination and/or warning coloration, offense via prey attraction and/or prey stunning with illumination, and lastly intraspecific communication for mate-finding purposes (Haddock *et al.*, 2010). The functions provided by bioluminescence may even change over the course of an individual's life history (Widder, 2010). This wide range in functional value

remains the reasoning as to why bioluminescence has evolved independently and repeatedly across so many taxa (Herring and Morin, 1978; Davis *et al.*, 2016).

Focusing on fishes specifically, bioluminescent species have been observed in a minimum of 42 families within 11 orders of the Class Actinopteri (ray-finned fishes) as well as two families of sharks (Haddock *et al.*, 2010; Claes *et al.*, 2015). Of these taxa, the majority produces luminous light intrinsically (Mallefet and Shimomura, 1995). Intrinsic luminescence is the production of light by the animal itself rather than through a symbiotic relationship with a luminous organism (Haddock *et al.*, 2010).

Although most luminous taxa carryout intrinsic luminescence, bioluminescent symbiosis has been observed in over 460 species of marine fishes across 21 families (Munk, 1999; Pietsch, 2009; Hendry and Dunlap, 2014). All bioluminescent symbionts identified within fishes belong to the family *Vibrionaceae* (Dunlap and Urbanczyk, 2013). Again, the evolution of such relationships are likely due to the beneficial functions such as prey and mate attraction provided by the luminous symbionts to the host (Herring and Morin, 1978) as well as the supply of potentially rare nutrients from host to symbiont (Haygood, 1993).

Anglerfishes

Of the vast array of deep-pelagic organisms, few are as captivating and mysterious as the deep-sea ceratioid anglerfishes. The ceratioids belong to the order Lophiiformes. Nearly all members of this order exhibit a uniquely modified first dorsal-spine, called the illicium, which is located on the snout, forehead or neck region and acts as a luring device used for the attraction of prey. Of the five suborders within Lophiiformes, the deep-sea ceratioids are the most phylogenetically derived and constitute the most species-rich vertebrate taxon within the bathypelagic zone, as new species are continually being discovered (Pietsch, 2009; Pietsch and Sutton, 2015).

Members of Ceratioidei differ remarkably from their less-derived, bottom-living relatives by having an extreme sexual dimorphism and unique mode of reproduction where the dwarfed males of some families may either temporarily or permanently attach themselves to the bodies of the females (Pietsch, 2009). Even more interesting, most female ceratioids possess a bioluminescent bacterial light organ at the distal tip of the

illicium. This light organ is called an "esca." The escal pigmentation, shape, orientation of appendages and/or filaments, and even size varies wildly across species (Pietsch, 2009). In fact, the morphological appearance of the esca has proven to be species specific. For this reason, differences in escal morphology have been the primary basis on which new ceratioid species are described (Pietsch, 2009). However, recently mitogenomic approaches have been used to extrapolate the evolutionary history and phylogenetic relationships of this diverse order (Miya *et al.*, 2010).

Females belonging to nine of the 11 families within the suborder Ceratioidei develop a bioluminescent lure which contains bacterial symbionts (Leisman *et al.*, 1980). Bioluminescent ceratioids use luminous symbionts to produce their characteristic glow. It is believed that anglerfishes are capable of controlling the bacterial populations by altering the conditions within their escae (Pietsch, 2009).

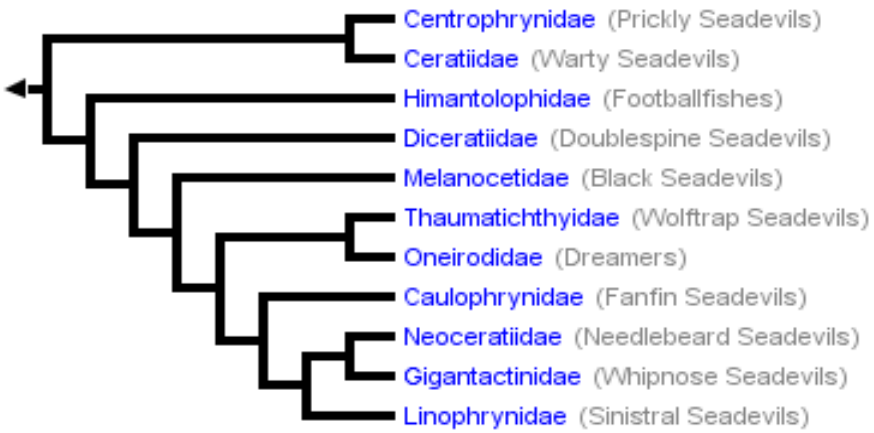


Figure 1. Phylogenetic tree of the suborder Ceratioidei (Pietsch and Kenaley, 2007).

The internal morphology of the esca is just as complex if not more complex than its outward appearance. In the most basic sense, the esca is composed of a spherical, bacteria-filled organ that contains a small opening to the external environment. However, that is not to imply that these organs are simple as they can also contain lenses, filters, and reflectors as noted previously regarding the photophores of non-symbiotic bioluminescent fishes (Munk, 1999). It is believed that these lures may be used for mate-finding purposes in addition to prey attraction (Herring, 2000, 2007). However, there still

remains much speculation regarding “who” their bioluminescent symbionts are and how they are acquired.

Previous studies indicate that the symbionts contained within anglerfish escae are unculturable via traditional laboratory techniques so sequencing methods were used by Haygood *et al.* in 1992. Their analysis of the full 16S rRNA gene for two ceratioid species indicated these symbionts are members of Vibrionaceae but are divergent from other known luminous symbionts. Their analysis concluded that the ceratioid symbionts may represent a new bacterial taxa and that the differences between the sequences obtained from each symbiont suggest they represent two separate bacterial species (Haygood *et al.*, 1992; Haygood and Distel, 1993).

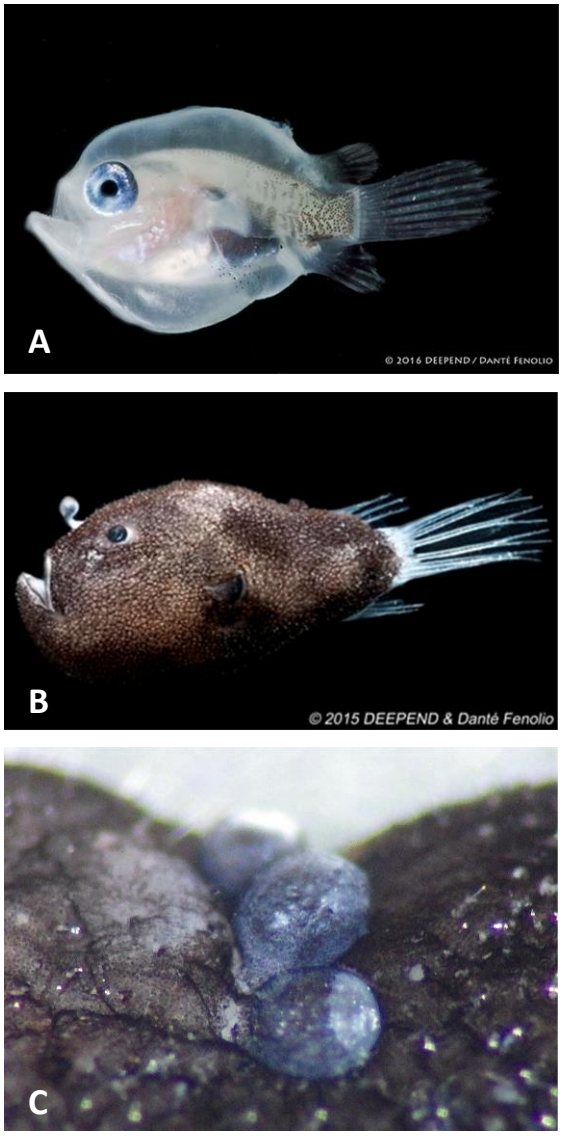


Figure 2. Bioluminescent organs of *C. couesii.* A) Larval *C. couesii* B) Adult *C. couesii* with arrows indicating the location of esca and caruncles C) Magnification of *C. couesii* caruncles (Photo of caruncles by Dr. Jon Moore)

Previous work suggested ceratioid symbionts were unculturable and potentially engaged in an obligate relationship with their hosts (Haygood and Distel, 1993) rather than a facultative relationship as recorded for most other marine bioluminescent symbionts (Dunlap and Urbanczyk, 2013). However, typically when an obligate bioluminescent symbiosis has been established, the symbiont is then transmitted from the parent generation to the offspring, as the symbiont is

dependent upon the host for growth (Dunlap *et al.*, 2007). Such a transmission pathway is not obviously evident based on the life cycle and escal morphology of ceratioids.

Larval anglerfish do not possess a lure capable of housing symbiotic bacteria (Munk and Herring, 1996). It is not until the larvae metamorphose as they make an ontogenetic vertical migration to the depths does the primordial esca invaginate to create a vacuole capable of holding bacteria (Munk *et al.*, 2009; Pietsch, 2009). However it has also been proposed that the female anglerfish may inoculate her eggs with the symbiont before the absorbent and buoyant egg raft makes its way towards the ocean surface where the larvae will hatch (Pietsch, 2009; Fukui *et al.*, 2010; Dunlap *et al.*, 2014). Lastly, the morphology of the lure implies that symbionts are exposed to the external environment via a pore opening (Munk, 1999).

In addition to the esca, several species of ceratioids have additional bioluminescent structures. Females within the families Ceratiidae and Diceratiidae possess a structure similar in form to the esca, which develops on the tip of the second dorsal spine. In larval ceratiids the escal-like organ lies externally just behind the primordial esca, but during metamorphosis sinks beneath the skin until eventually losing connection to the second dorsal spine and external environment. Meanwhile in diceratiid larvae, the escal-like organ forms at the tip of a short stalk just behind the illicium and remains connected to the second dorsal-fin spine and external environment even through adulthood. Ceratiids also possess an escal-like, modified anterior dorsal-fin ray. Members of the genus *Ceratias* display two such organs (referred to as caruncles), while members of the genus *Cryptopsaras* have three caruncles. Unlike the modified second dorsal spines, which have not been found to contain bioluminescent bacteria, histological study of *C. couesii* caruncle has concluded that dense populations of luminous bacteria are present within the caruncle and can be expelled through a distal pore (Hansen and Herring, 1977; Herring and Morin, 1978).

Lastly, bioluminescence has also been observed in the hyoid barbels of metamorphosed females belonging to the genus *Linophryne.* However, unlike the esca and caruncles, histological study of the barbels has revealed that bioluminescence within the hyoid barbels of the genus *Linophryne* is done intrinsically via photophores rather than through the use of symbiotic bacteria (Hansen and Herring, 1977).

Microbiome Characterization

Although luminous bacteria are of great interest within the depths of the ocean, microbes in general are present at astounding numbers within seawater and play an essential role in the planet's ecosystems (Pedros-Alio, 2006; Logares *et al.*, 2012). However, it has long been recognized that the majority of microorganisms cannot be readily cultured in a laboratory setting (Bruns *et al.*, 2002; Knight *et al.*, 2012).

With the more recent development of affordable 16S rRNA high-throughput sequencing (HTS) technologies, microbes can be identified with little to no knowledge of their morphology or physiology. This technique has proven very useful for the characterization of microbial communities, also referred to as microbiomes (44–47). Through these methods, we are now able to measure entire microbial assemblages or even host-specific correlations that might otherwise be missed in studies of an individual microbial species (Bartram *et al.*, 2011; Knight *et al.*, 2012).

The ribosomal RNA (rRNA) molecule is generally accepted as a universal and comparative molecule for microbial phylogenetic and taxonomic analysis (Janda and Abbott, 2007). This is due to the fact that the rRNA molecule is present in almost all bacteria and is part of a large complex that is vital for cell function. Since it is functionally important and highly conserved, 16S rRNA sequencing allows for reliable phylogenetic comparisons between microbial organisms (Janda and Abbott, 2007). The 16S gene is also useful for taxonomic study because it is not necessary to sequence the full gene to discriminate between taxa. The 16S gene is comprised of nine hypervariable regions (V1-V9) (Tringe and Hugenholtz, 2008; Wang and Qian, 2009). The V3-V4 regions have been shown to generate the most accurate taxonomic results when paired with the longer read lengths of the Illumina high-throughput sequencing technologies (Vasileiadis *et al.*, 2012; Fadrosh *et al.*, 2014). However, this approach does lead to weakened phylogenies at the species level. For more accurate results at the species level, the full 16S gene should be sequenced (Janda and Abbott, 2007; Birtel *et al.*, 2015).

Hypotheses

The objective of this study is to build upon previous work on the bioluminescent symbionts of ceratioid fishes by characterizing the escal microbiome via high-throughput sequencing techniques. Sequencing results will then be analyzed to identify potential symbiont taxa and compare their relative abundance across anglerfish organs and seawater samples in an effort to resolve whether parent to offspring trasmission or environmental acquisision is more plausible.

Hypothesis 1

The relative abundance of potential symbiont OTUs will be significantly greater in escal samples of adult hosts as compared to other organ types from adults of the same host species.

Hypothesis 2

The potential symbiont OTUs identified within the escal samples of adult hosts will be present within DEEPEND GOM seawater samples.

Hypothesis 3

Potential symbiont OTUs will continue to exhibit host specificity at the family level and potentially the species level with the inclusion of additional host specimens from the same genus.

Hypotheses 4

The potential symbiont OTUs identified within the escal samples of adult hosts will also be present in larval anglerfishes of the same species.

Significance

To date, the luminous symbionts of only two ceratioid species have been examined using sequencing methods (Haygood *et al.*, 1992; Hendry *et al.*, 2018). Due to the depths at which these organisms live, it is difficult to gather samples. This study will be the most comprehensive examination to date of ceratioid symbionts via molecular

methods. This study differs from the work previously done on this topic in that it proposes to examine the entire microbial community present within the luminous esca, as well as on the skin, gills, fins, guts, and caruncles of adult anglerfishes in addition to the primordial escae of larval anglerfishes. This study also investigates the presence of the identified escal symbionts within Gulf seawater in order to gain some clarity on the potential mode of symbiont transmission. Understanding these symbiotic relationships may provide insight as to whether future anthropogenic impacts to the deep pelagial may pose a threat to their continuation.

MATERIALS AND METHODS

Sample Collection and Processing

All anglerfish and seawater samples were collected over the course of four cruises aboard the *R/V Point Sur* in the Gulf of Mexico: DP01 from May 1 – 8, 2015, DP02 from

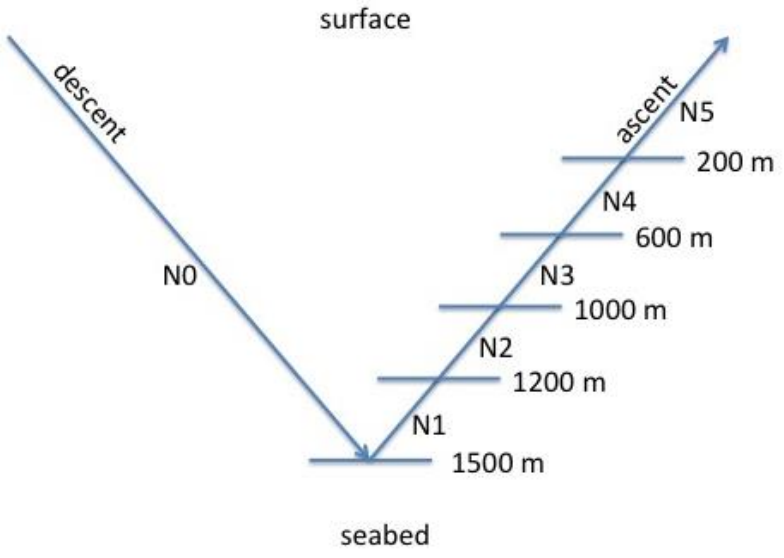


Figure 3. MOC-10 Sampling Profile

August 8-21, 2015, DP03 from April 20 – May 14, 2016, and DP04 from August 5-19, 2016. Previously established SEAMAP station locations were used for labeling collection sites (www.gsmfc.org). All anglerfish specimens were collected using a 10 m^2 mouth

area, six-net MOCNESS (Multiple Opening and Closing Environmental Sensing System) with 3-mm mesh (Wiebe *et al.*, 1976).

Table 1. Anglerfishes collected for microbiome analysis. Abbreviations for sampled organs: caruncle (c), esca (e), fins (f), illicium (i), gills (g), guts (gu), and/or skin (s).

ID	**Taxonomy (Family, species)**	**Dev. Stage**	**Organs sampled**	**Cruise**	**Station**	**Trawl #**	**Trawl Depth (m)**
DP02	Oneirodidae *Dolophichys* sp.	Adult	e, g, gu, s	DP01	B001	02	0-1201
MJ02	Melanocetidae *Melanocetus johnsonii*	Adult	e, f, g, gu, s	DP01	B001	03	0-1143
CC24	Ceratiidae *Cryptopsaras couesii*	Adult	e	DP02	B252	24	600-198
CC26	Ceratiidae *Cryptopsaras couesii*	Adult	e	DP02	B080	26	0-751
CC32	Ceratiidae *Cryptopsaras couesii*	Adult	e	DP02	SE3	32	597-198
CC34	Ceratiidae *Cryptopsaras couesii*	Adult	e	DP02	B255	34	1000-600
CC42	Ceratiidae *Cryptopsaras couesii*	Larva	c, e, s	DP03	B003	42	998-599
CC53.N0	Ceratiidae *Cryptopsaras couesii*	Adult	e	DP03	B081	53	11-1504
CC53.N3	Ceratiidae *Cryptopsaras couesii*	Adult	e, i	DP03	B081	53	1002-601
CU44	Undefined *Ceratias* sp.	Adult	e, i	DP03	B079	44	997-601
CU51	Undefined *Ceratias* sp.	Adult	e	DP03	B252	51	11-1502
MM54	Melanocetidae *Melanocetus murrayi*	Adult	e, i	DP03	B081	54	11-1500
CC57	Ceratiidae *Cryptopsaras*	Adult	c, e, f, g, gi, s	DP04	SW6	57	10-924

	couesii						
LI58	Unknown Linophrynidae sp.	Larva	e, s	DP04	SW6	58	1515-1203
CC59	Ceratiidae *Cryptopsaras couesii*	Larva	e	DP04	SW6	59	202-10
GI59	Unknown Gigantactinidae sp.	Larva	e, s	DP04	SW6	59	10-1500
LI59	Unknown Linophrynidae sp.	Larva	e, s	DP04	SW6	59	1498-1201
CC60	Ceratiidae *Cryptopsaras couesii*	Larva	c, e, f, g, gu, s	DP04	SW4	60	999-602
CS60	Centrophrynidae *Centrophryne spinulosa*	Adult	e, i	DP04	SW4	60	999-602
ON62.1	Unknown Oneirodidae sp.	Larva	e, s	DP04	SE1	62	11-1499
CC62	Ceratiidae *Cryptopsaras couesii*	Adult	c, e, i, f, g, gu, s	DP04	SE1	62	11-1499
ON62.2	Unknown Oneirodidae sp.	Larva	e, s	DP04	SE1	62	11-1499
ON64	Unknown Oneirodidae sp.	Larva	e, s	DP04	SE3	64	11-1501
ON69	Unknown Oneirodidae sp.	Larva	e, gu, s	DP04	SW3	69	998-601
CC70	Ceratiidae *Cryptopsaras couesii*	Adult	c, f, g, gu, s	DP04	SW5	70	998-600
CC71.N0	Ceratiidae *Cryptopsaras couesii*	Adult	c, e, f, g, gu, i, s	DP04	SW5	71	11-1505
CC71.N3	Ceratiidae *Cryptopsaras couesii*	Adult	c, e, f, g, gu, i, s	DP04	SW5	71	1001-593
CC73	Ceratiidae *Cryptopsaras couesii*	Adult	e, f, g, gu, i, s	DP04	B064	73	11-1512
ON76	Unknown Oneirodidae sp.	Post Larva	e, f, g, gu, s	DP04	B065	76	1000-599
LI78	Unknown Linophrynidae	Larva	e, s	DP04	B287	78	996-603

	sp.						
ON78	Unknown Oneirodidae sp.	Larva	e, s	DP04	B287	78	11-1501
CC79.1	Ceratiidae *Cryptopsaras couesii*	Adult	c, e, f, g, gu, i, s	DP04	B252	79	1001-605
CC79.2	Ceratiidae *Cryptopsaras couesii*	Adult	c, e, f, g, gu, s	DP04	B252	79	1001-605
CC80	Ceratiidae *Cryptopsaras couesii*	Adult	e	DP04	B252	80	10-1500
CC81	Ceratiidae *Cryptopsaras couesii*	Adult	c, e, f, g, gu, s	DP04	B175	81	1000-600

Water samples were also collected at each station using a separate CTD cast. During each cast, Niskin bottles were fired at a maximum of five targeted depths based on depth, chlorophyll *a* fluorescence, or dissolved oxygen levels. Four to five liters of seawater were collected from each sampled depth and separated into three one-liter replicates that were then filtered through a 0.45-micron filter (Daigger) under low pressure using a vacuum pump (Easson and Lopez, 2018, in review).

Table 2. Water samples collected for microbiome analysis.

Cruise	**CTD Cast #**	**Station**	**Depth(m)**
DP01	1	B001	1000, 450, 50, 2
DP01	2	B175	1000, 450, 2
DP01	3	B175	75, 35
DP01	4	B252	400, 30
DP01	5	B287	1600, 475
DP01	6	B287	95, 75
DP01	7	B082	1600, 465, 65
DP01	8	B250	1600, 1000, 450, 75
DP02	9	SW4	1466, 600, 130, 1
DP02	10	SW4	1500, 650, 110, 1
DP02	13	SE1	1500, 750

DP02	14	B286	1490, 660
DP02	16	B287	1507, 467, 90, 1
DP02	17	B252	1500, 462, 70, 1
DP02	18	B175	1500, 1404, 40, 1
DP02	19	B175	1404, 399, 1
DP02	20	B080	800, 498, 73, 1
DP02	21	B080	800, 500, 43, 12
DP02	22	B003	1510, 457, 72, 1
DP02	24	B079	1510, 600, 92, 1
DP02	27	SE4	1499
DP02	28	SE4	1500
DP02	29	B255	1496
DP02	30	B255	1500
DP03	31	B082	1600, 456, 80
DP03	32	B082	1600, 450, 80, 2
DP03	33	B082	1500, 377, 68, 2
DP03	34	B082	1600, 375, 50, 2
DP03	35	B287	1500, 303, 56, 2
DP03	36	B287	1500, 283, 160, 52, 2
DP03	37	B287	274, 245, 50
DP03	38	B003	1500, 244, 59, 2
DP03	39	B003	300, 50
DP03	40	B003	1500, 252, 64, 2
DP03	41	B079	1500, 237, 70, 2
DP03	42	B079	1500, 347, 94, 2
DP03	43	B079	1500, 360, 86, 2
DP03	44	B079	300, 50
DP03	45	SE4	1500, 533, 145, 105, 2
DP03	46	SE4	300, 50
DP03	47	SE5	1500, 511, 106, 2
DP03	48	B252	396, 64, 2
DP03	49	B252	360, 49, 2
DP03	50	B081	1500, 467, 49, 2
DP03	51	B081	1500, 480, 53, 2

DP03	52	B175	1500, 485, 54, 2
DP03	53	B175	507, 59, 2
DP04	54	SW6	1499, 545, 130, 2
DP04	55	SW6	1502, 516, 125, 2
DP04	56	SW4	1500, 446, 43, 2
DP04	57	SE1	1495, 441, 68, 2
DP04	58	SE3	1501, 444, 90, 2
DP04	59	SE3	1500, 418, 86, 2
DP04	60	SE2	1500, 386, 86, 2
DP04	61	SW3	1500, 359, 76, 2
DP04	62	SW5	1500, 498, 110, 2
DP04	63	B064	1520, 421, 97, 2
DP04	64	B064	1500, 415, 95, 22, 2
DP04	65	B065	1500, 334, 58, 2
DP04	66	B287	1503, 340, 70, 2
DP04	67	B252	1501, 415, 80, 2
DP04	68	B175	1500, 374, 51, 2

All specimens were stored at -80C until processed by the Microbiology & Genetics Laboratory at Nova Southeastern University's Halmos College of Natural Sciences and Oceanography. Reports for each of the four cruises can be found at the following sites: http://www.deependconsortium.org/images/documents/DP01_report.pdf, http://www.deependconsortium.org/images/documents/DP02_CruiseReport.pdf, http://www.deependconsortium.org/images/documents/DP03_CruiseReport.pdf, and http://www.deependconsortium.org/images/documents/DP04_Cruise_Report.pdf.

Specimen Taxonomy

Once onboard, anglerfish specimens were sorted, identified to the lowest taxonomic level possible, and placed in ethanol or RNALater by DEEPEND Consortium's Chief Scientist Dr. Tracey Sutton (Sutton *et al.*, 2010; Pietsch and Sutton, 2015).

Microbial DNA Extraction

Anglerfish specimens were dissected with sterilized instruments. For specimens collected during cruises DP01 and DP02, the entiring luring apparatus (esca and illicium) were dissected as a single sample labeled as esca. Lure samples collected during the later cruises (DP03 and DP04), were split into two separate specimens labeled as the esca and illicium accordingly. For Ceratiid specimens, the base of the caruncles was separated from the back of the fish and all two or three caruncles, depending on anglerfish species, were included in the sample. The least damaged pectoral fin was dissected as well as an undamaged portion of skin from the lateral side of the anglerfishes. For gill sample dissection, the gill-filaments, gill-rakers, and gill arch were removed from one side of the anglerfish. Lastly, the entire intestine, from the base of the stomach to the cloaca was extracted for the gut sample.

All microbial DNA isolations were conducted following the Earth Microbiome Project (earthmicrobiome.org) protocol with the MO BIO PowerLyzer™ PowerSoil® kit. After extraction, a 1% agarose gel was run to ensure that the DNA extraction was successful. After gel verification, the DNA concentration was confirmed using the Qubit 2.0 (Life Technologies).

Illumina High-Throughput Metagenomic Sequencing

All samples were prepared for sequencing following the 16S Illumina Amplicon Protocol per the Earth Microbiome Project (Caporaso *et al.*, 2011). The 806R and 515F primers were used for PCR amplification of the V4 region of the 16S rRNA gene (Caporaso *et al.*, 2011). Amplicons were sequenced with an Illumina MiSeq using the V2 500-cycle cartridge across three runs to generate paired-end 250 base pair amplicons (Caporaso *et al.*, 2012).

Sequencing Analysis: QIIME

The initial processing of raw microbiome data was performed using Quantitative Insights into Microbial Ecology (QIIME) version 1.9.1 (Caporaso *et al.*, 2010). The forward and reverse paired-end reads were joined and converted to FASTA files using "join_paired_ends.py" with the default settings. Sequences were then demultiplexed and

quality filtered (quality score > 29) using "split_libraries_fastq.py." Lastly, sequences were clustered into operational taxonomic units (OTUs) based on 97% similarity using the default settings for "pick_open_reference_otus.py." Taxonomic classification was assigned via the GreenGenes database (DeSantis *et al.*, 2006; Caporaso *et al.*, 2010).

Community Analysis: R

Analysis was executed with the RStudio software (version 3.2.1, (R Core Team, 2016), with the added packages 'phyloseq' and 'vegan' to examine general microbial ecology (McMurdie and Holmes, 2013; Oksanen *et al.*, 2018). Seawater replicates were merged into a single sample per collection depth and location. All samples were then rarefied to a uniform depth of 1000 sequences and were transformed to reflect relative abundance. Variations associated with sample type (anglerfish or water), organ type (esca, caruncle, illicium, fin, gill, gut, or skin), anglerfish developmental stage (larval, post-larval, or adult) were analyzed using these tools.

Alpha diversity was measured by calculating OTU observed richness, Chao1 index, Shannon index, and the Inverse Simpson's index for each sample type, anglerfish organ type, and anglerfish developmental stage using phyloseq (McMurdie and Holmes, 2013). Differences in alpha diversity among sample type, organ type, and developmental stage were assessed using an analysis of variance (ANOVA) followed by the post hoc test, Tukey's Honest Significant Difference (HSD) to determine pairwise differences.

Beta diversity was measured by calculating Bray-Curtis dissimilarity to determine differences in the community composition by sample type, anglerfish organ type, and anglerfish developmental stage. Dissimilarity was presented as distance matrices and a permuted multivariate ANOVA (Adonis) was used to assess significant differences. Lastly, a SIMPER test with 499 permutations was used to show which specific taxa were driving differences between sample type and organ type microbiomes.

Symbiont Analysis: R

For symbiont analysis, the original, unrarefied dataset was used so as not to exclude rare taxa that may have been inadvertently excluded when normalizing to a uniform depth of 1000 sequences. For this dataset, 16S rRNA sequence data was

transformed to reflect relative abundance. The most abundant OTUs (relative abundance >10%) were examined within escal and caruncle samples of adult anglerfish samples to identify potential bioluminescent symbiont taxa. These were then filtered for members belonging to the family *Vibrionaceae*, which contains known bioluminescent symbionts of fishes (Dunlap and Urbanczyk, 2013). A phylogenetic tree for the most abundant OTUs (relative abundance >10%) was also generated to verify that any taxa not classified to the family level were not excluded unintentionally (Supplemental Figure 13). Once potential bioluminescent symbiont taxa were identified within adult anglerfish samples, larval anglerfish samples of matching species were examined for identical OTUs. The same process to identify potential symbionts in the adult anglerfish samples was used to identify additional potential symbionts within larval specimens for which an adult specimen of the same species was not available. Lastly, the relative abundance of these potential symbiont taxa was determined within other anglerfish organ types and within water samples.

RESULTS

Microbiome samples

Following pre-processing, a total of 330 samples were analyzed, including 116 anglerfish samples and 214 seawater samples. Anglerfish samples comprised the esca of 21 adults and 13 larvae, caruncles of nine adults and two larvae, illicium of 10 adults, skin of 11 adults and 12 larvae, fins of 10 adults and two larvae, gills of 11 adults and two larvae, and finally the guts of 10 adults and three larvae. Anglerfish samples were collected from 36 individuals belonging to six families within the suborder Ceratioidei (Table 1). Each family was represented by one - 19 individuals. While taxonomic identification was based upon morphology for this study, there is an ongoing effort by the DEEPEND Consortium to also determine the taxonomy of each specimen based on CO1 gene barcoding.

Sequencing results

A total of 64,145,146 MiSeq reads and 192,860 OTUs were generated across all 734 samples included in this study. Of these, 6,876,285 MiSeq reads were generated from

the 117 anglerfish samples while 57,268,861 MiSeq reads were generated from 617 water samples. The mean read depth for all samples was 87,391. The mean read for water samples was 92,818 and for anglers was 58,771 (Supplemental Table 1). For the water samples, replicates were merged into a single sample resulting in a total of 214 merged water samples (Table 2). Samples with fewer than 1000 sequences were excluded due to inadequate sequencing depth resulting in a final count of 330 samples (Supplemental Table 1). In total, 14,947 microbial OTUs (97% similarity clusters) were recovered across all samples after rarefaction to a common sequence count of 1000.

Due to the rarity and scientific value of the Ceratioidei specimens, collection of identical adult and larval sample sets was not possible. Adult individuals from four of six families (Oneirodidae, Ceratiidae, Melanocetidae, and Centrophrynidae) were collected while larvae from families Oneirodidae, Ceratiidae, Linophrynidae, and Gigantactinidae were collected. Due this uneven sampling across host family, general comparisons of the microbial communities belonging to adult and larval anglerfishes should be done with caution as differences may be biased by host taxonomic composition.

Comparison of Anglerfish and Water Microbiomes

Alpha and beta diversity varied significantly between anglerfish-associated samples and seawater samples. There was a significant difference between the water and anglerfish samples by observed richness (ANOVA, df=1, F=449.9, p=<0.001), Chao1 index (ANOVA, df=1, F=276.6, p=<0.001), Shannon index (ANOVA, df=1, F=560.7, p=<0.001), and the Inverse Simpson index (ANOVA, df=1, F=127.2, p=<0.001). Anglerfish samples had significantly less microbial richness and microbial diversity than water (Figure 3). While anglerfishes and their environment shared some taxa (13.2% of OTUs), they had fairly distinct microbial communities (Figure 4). NMDS analysis and visualization of the data by sample type (Anglerfish or Water) revealed a distinct clustering of water samples while anglerfish samples were more variable (Figure 4). Adonis showed that the interaction between sample types (Anglerfish or Water) had a moderate impact on the differences between groups as it explained only 13% of the variation (PERMANOVA, df=1, F=49.59, R^2=0.13, p=0.001).

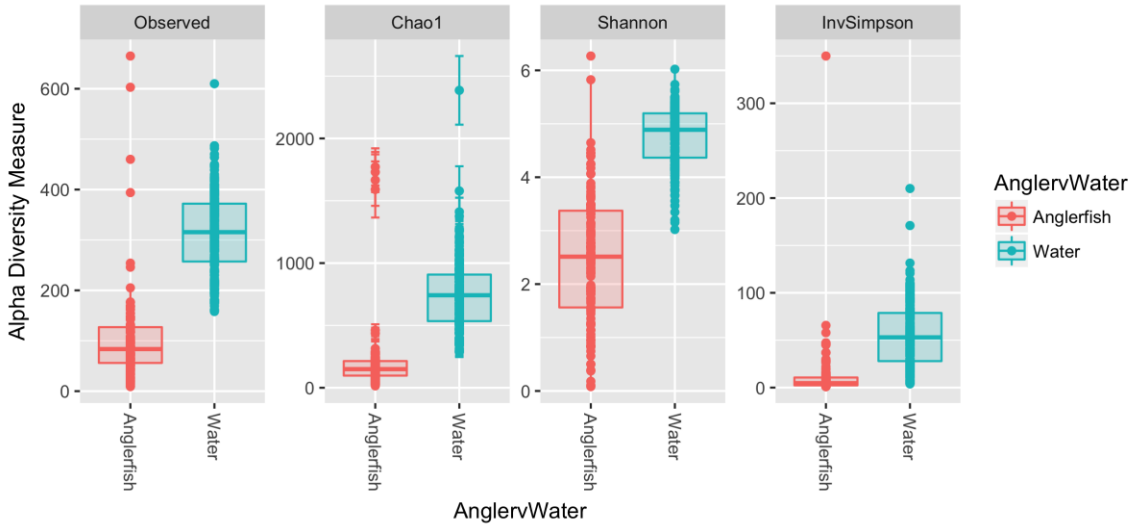


Figure 4. Boxplot of species richness and diversity comparing anglerfish samples to water samples based on observed richness (ANOVA, df=1, F=449.9, p=<0.001), Chao1 index (ANOVA, df=1, F=276.6, p=<0.001), Shannon index (ANOVA, df=1, F=560.7, p=<0.001), and Inverse Simpson index (ANOVA, df=1, F=127.2, p=<0.001).

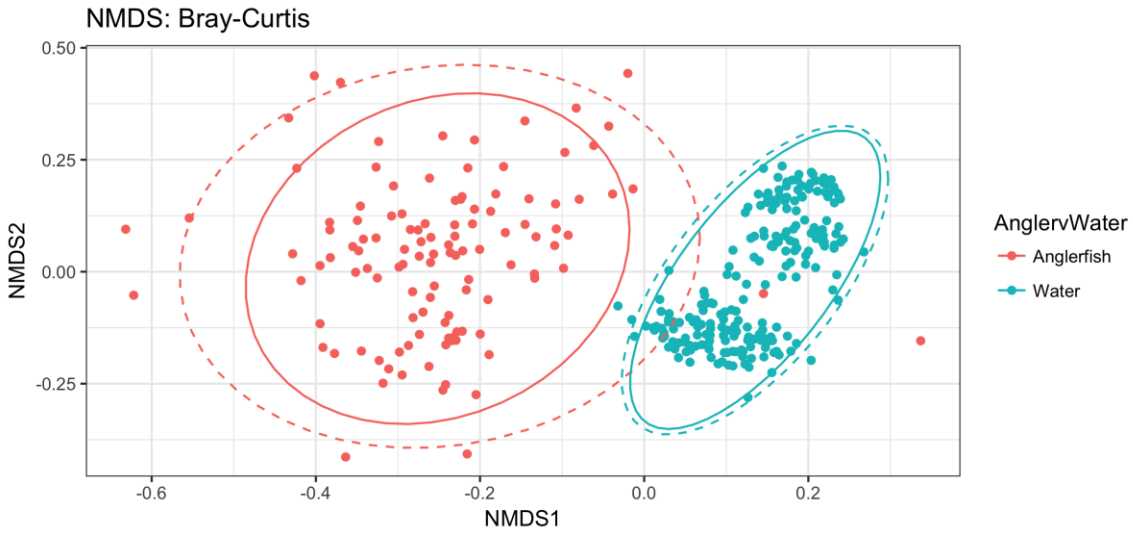


Figure 5. Non-metric dimensional scaling of anglerfish and water samples. (R^2 = 0.97, stress= 0.1695, dashed ellipse = multivariate t distribution with 95% CI, solid ellipse = multivariate normal distribution with 95% CI).

SIMPER analysis revealed that OTUs 112983 (*Moritella* sp.), 830290

(*Pseudoalteromonas* sp.), 9131 (*Enterovibrio* sp.), and 792393 (*Vibrio shilonii*) were driving the significant differences between anglerfish and water microbiomes accounting for 15.5%, 9.5%, 8.8%, and 6.7% of the differences respectively.

Anglerfish specimens were also examined by organ type in comparison to each other and to the water samples. Significant differences were found in the microbial community richness and diversity (Figure 5). The observed richness (ANOVA, df=7, F=68.15, p=<0.001) and Chao1 index (ANOVA, df=7, F=40.76, p=<0.001) showed significant differences in richness and diversity among sample types. Diversity as measured by the Shannon index (ANOVA, df=7, F=89.5, p=<0.001) and InvSimpson index (ANOVA, df=7, F=20.51, p=<0.001) also showed significant differences among sample types. The significant results were mainly driven by differences between the anglerfish samples compared to the water. NMDS analysis and visualization of the data again revealed a distinct clustering of water samples while all anglerfish organ types overlapped (Figure 6). Adonis showed that examining anglerfish specimens at the organ level to water provided a slightly greater explanation as this accounted for 17% of the variation (PERMANOVA, df=7, F=9.09, R^2=0.17, p=0.001).

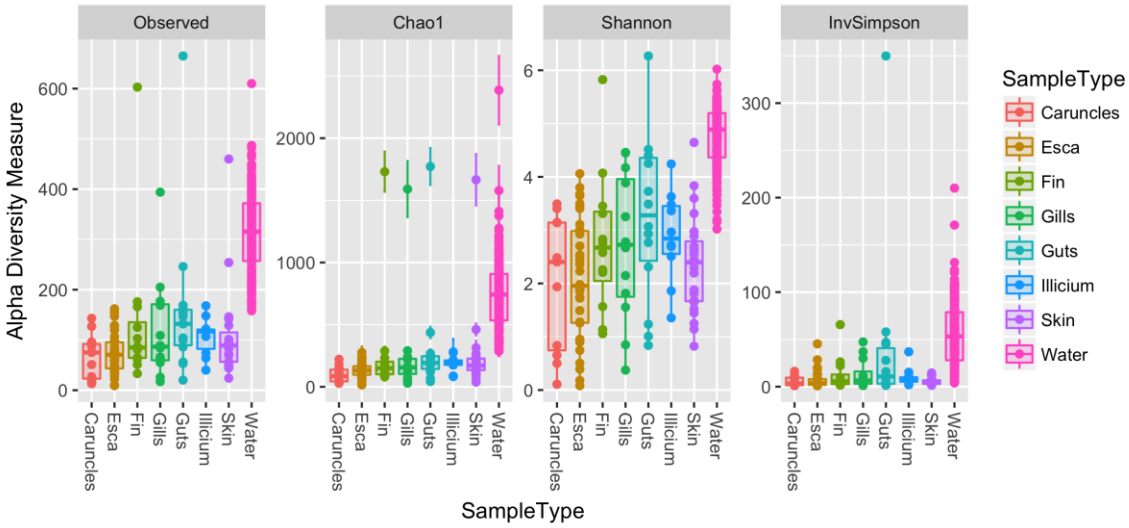


Figure 6. Boxplot of species richness and diversity comparing sample types based on observed richness (ANOVA, df=7, F=68.15, p=<0.001), Chao1 index (ANOVA, df=7, F=40.76, p=<0.001), Shannon index (ANOVA, df=7, F=89.5, p=<0.001), and Inverse Simpson index (ANOVA, df=7, F=20.51, p=<0.001).

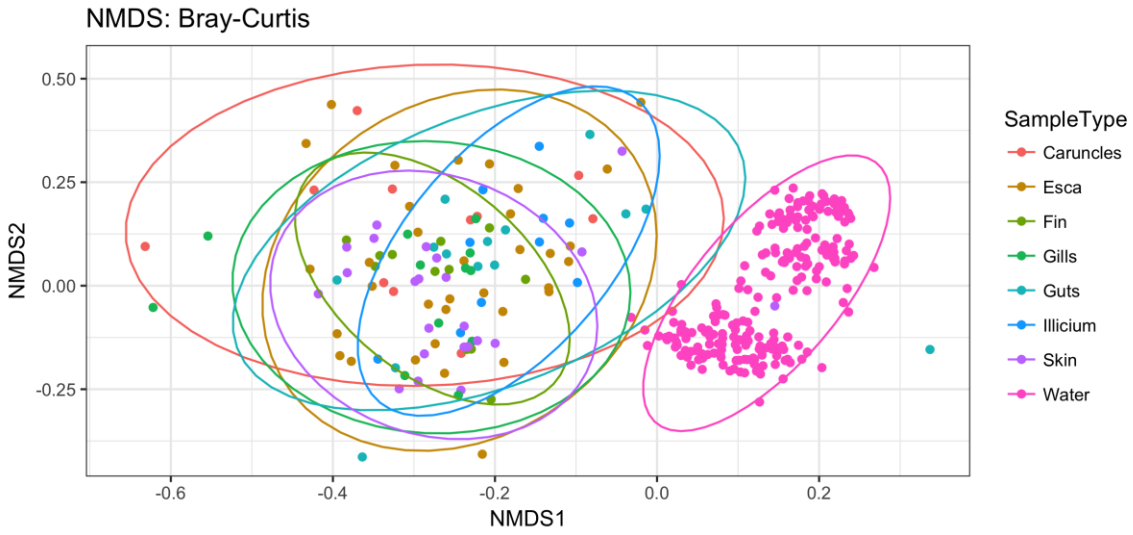


Figure 7. Non-metric dimensional scaling of anglerfish and water samples. (R^2 = 0.97, stress= 0.1699, solid ellipse = multivariate normal distribution with 95% CI).

Water samples were then excluded in order to directly compare the microbial richness and diversity of anglerfish organ types to one another. Significant differences in the microbial community richness and diversity were found between anglerfish organ types as measured by the Shannon index (ANOVA, df=6, F=2.204 p=0.048) and Inv. Simpson index (ANOVA, df=6, F=2.244, p=0.044). These significant results were driven by differences between the guts and esca, (InvSimpson, Tukey's HSD P=0.022), and between the guts and skin (Inv. Simpson, Tukey's HSD P=0.025).

Anglerfishes by Developmental Stage

No significant differences were found in the microbial community richness or diversity among anglerfishes of varying developmental stages (Figure 7). Neither observed richness (ANOVA, df=2, F=1.677, p=0.192), Chao1 index (ANOVA, df=2, F=1.06, p=0.35), Shannon index (ANOVA, df=2, F=1.036, p=0.358), nor InvSimpson index (ANOVA, df=2, F=0.438, p=0.646) showed significant differences in community richness or diversity among developmental stages. However, comparisons across developmental stages may be muddled by differences in anglerfish taxonomic composition.

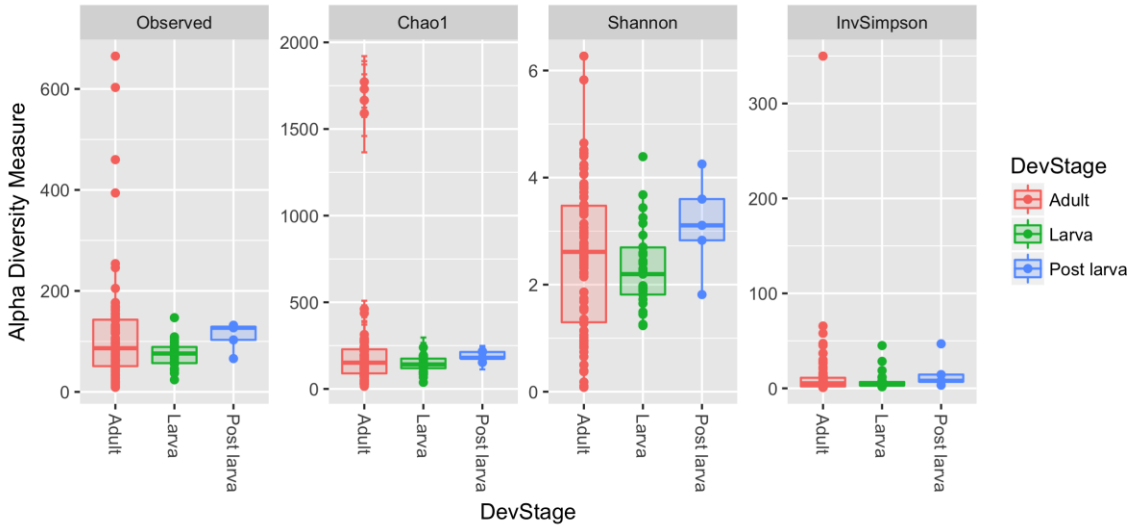


Figure 8. Boxplot comparing species richness and diversity of anglerfishes at various developmental stages. Observed richness (ANOVA, df=2, F=1.677, p=0.192), Chao1 index (ANOVA, df=2, F=1.06, p=0.35), Shannon index (ANOVA, df=2, F=1.036, p=0.358), and Inverse Simpson index (ANOVA, df=2, F=0.438, p=0.646).

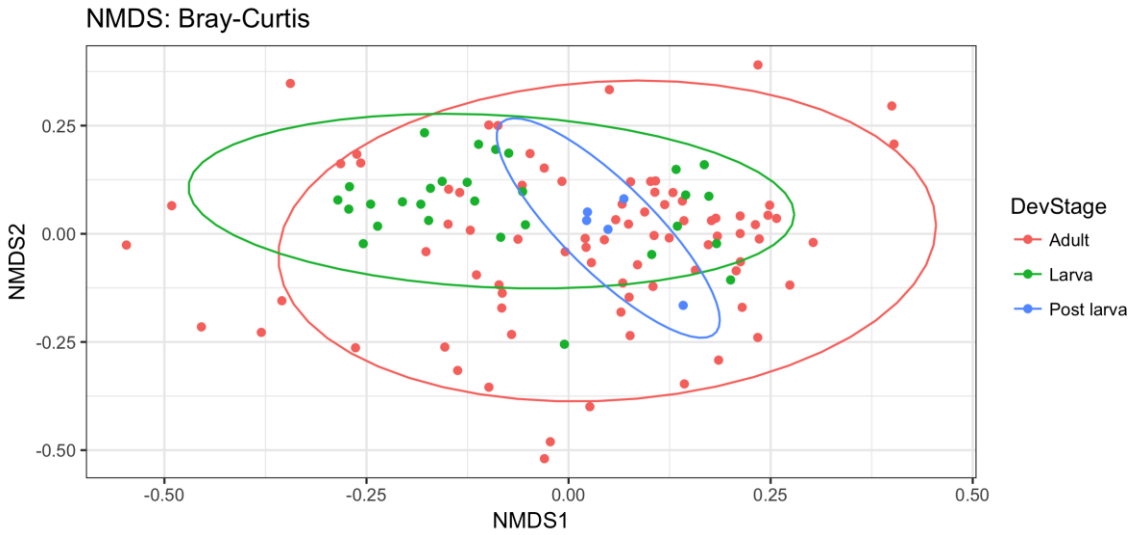


Figure 9. Non-metric dimensional scaling of anglerfish specimens by developmental stage. (R^2 = 0.95, stress= 0.2303, solid ellipse = multivariate t distribution with 95% CI).

Adult Anglerfish Samples

No significant differences were found in microbial community richness or diversity among adult anglerfish organ types as measured by observed richness (ANOVA, df=6, F=1.624, p=0.151), Chao1 index (ANOVA, df=6, F=1.086, p=0.378), Shannon index (ANOVA, df=6, F=1.907, p=0.0898), or Inverse Simpson index (ANOVA, df=6, F=1.597, p=0.159) (Figure 9). NMDS analysis and visualization of the data by organ type did not show any obvious clusters but did reveal similar orientation of the ellipses for the caruncle and escal organ types in comparison to all other organ types (Figure 10). Adonis showed that the interaction between organ types in adult anglerfish specimens had a moderate impact as it explained 14% of the variation (PERMANOVA, df=6, F=2.1292, R^2=0.1377, p=0.001). Although not significant, it was worth noting that the bioluminescent organs (esca and caruncle) overall had the lowest mean richness and diversity measurements (Supplemental Table 3).

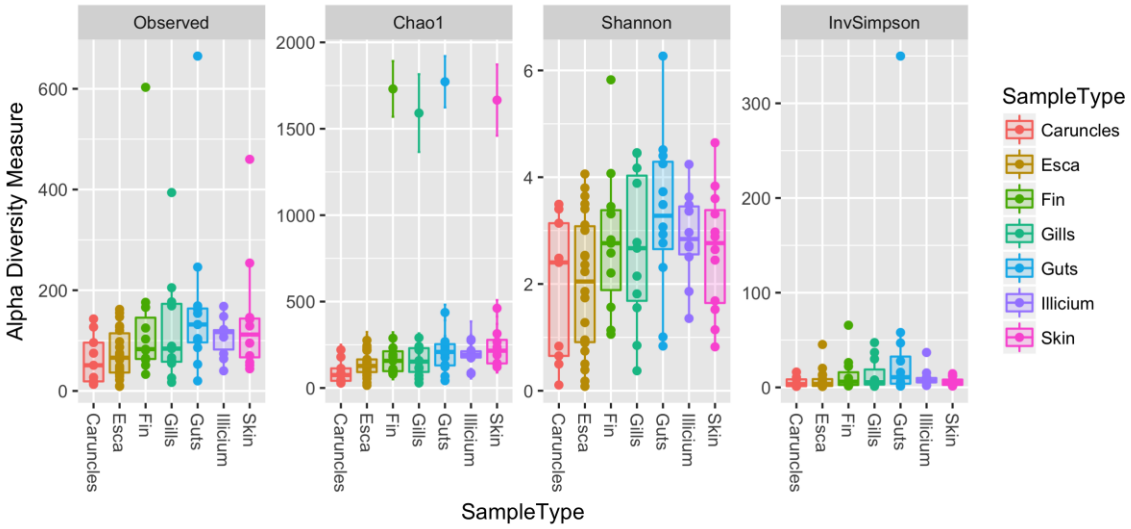


Figure 10. Boxplot of species richness and diversity by sample types in adult anglerfish specimens. Observed richness (ANOVA, df=6, F=1.624, p=0.151), Chao1 index (ANOVA, df=6, F=1.086, p=0.378), ANOVA, df=6, F=1.907, p=0.0898), and Inverse Simpson index (ANOVA, df=6, F=1.597, p=0.159).

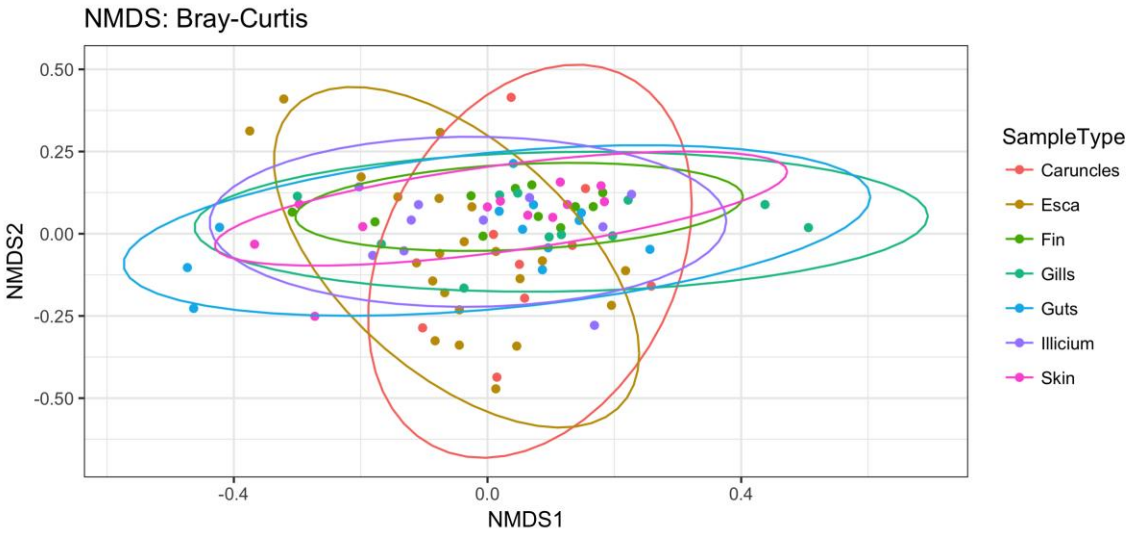


Figure 11. Non-metric dimensional scaling of adult anglerfish organ types (R^2 = 0.95, stress= 0.2246, solid ellipse = multivariate normal distribution with 95% CI).

Upon examining beta diversity by anglerfish species, anglerfish family, collection station, collection depth zone, and organ type, the Adonis test indicated that the collection station explained the greatest percentage of variation within the microbial community (PERMANOVA, df=13, F=3.36, R^2=0.374, p=.001). Collection station was followed by anglerfish species, sample type, anglerfish family, and collection depth zone, respectively (Supplemental R Code).

Larval Anglerfish Samples

No significant differences were found in microbial community richness or diversity among larval anglerfish organ types as measured by observed richness (ANOVA, df=5, F=1.028, p=0.42), Chao1 index (ANOVA, df=5, F=0.436, p=0.82), or Shannon index (ANOVA, df=5, F=0.854, p=0.524). However, the Inverse Simpson index (ANOVA, df=5, F=4.33, p=0.005) did indicate significant difference in diversity. The significant results were driven by differences between the guts and esca (InvSimpson, Tukey's HSD P=0.003), guts and fin (InvSimpson, Tukey's HSD P=0.0437), and guts and skin samples (InvSimpson, Tukey's HSD P=0.001) (Supplemental Table 5). NMDS analysis and visualization of the data by organ type did not show any obvious clusters which was supported by the Adonis test which indicated that the interaction between

organ types in larval anglerfish specimens was not significant (PERMANOVA, df=5, F=1.01, R^2=0.1528, p=0.456).

Examination of beta diversity by anglerfish species, anglerfish family collection, station, collection depth zone, and organ type revealed that the that the collection station explained the greatest percentage of variation within the microbial community of larval anglerfish specimens as well (PERMANOVA, df=13, F=3.36, R^2=0.374, p=.001). Collection station was followed by collection depth zone, anglerfish species, and anglerfish family, respectively.

Adult Anglerfish Symbiont Taxa

In order to identify potential bioluminescent symbionts within the adult anglerfish specimens, the unrarefied OTU table was transformed into relative abundance and filtered for OTUs which make up greater than 10% of the relative abundance within a sample. The most abundant families of microbes within adult anglerfish specimens were Vibrionaceae, Moritellaceae, Psuedoalteromonadaceae comprising 25.3%, 14.6%, and 7.79% relative abundance, respectively. Although most abundant overall, Vibrionaceae was primarily found within the caruncle and escal specimens but was not limited solely to the bioluminescent organs (Figure 11). Members of the family Moritellaceae were present in highest abundance on the fins, skin, and guts, while Pseudoalteromonadaceae was most abundant from the escae and illicia samples, which were not surface sterilized and so could be from either the internal or external regions of the escae (Figure 11).

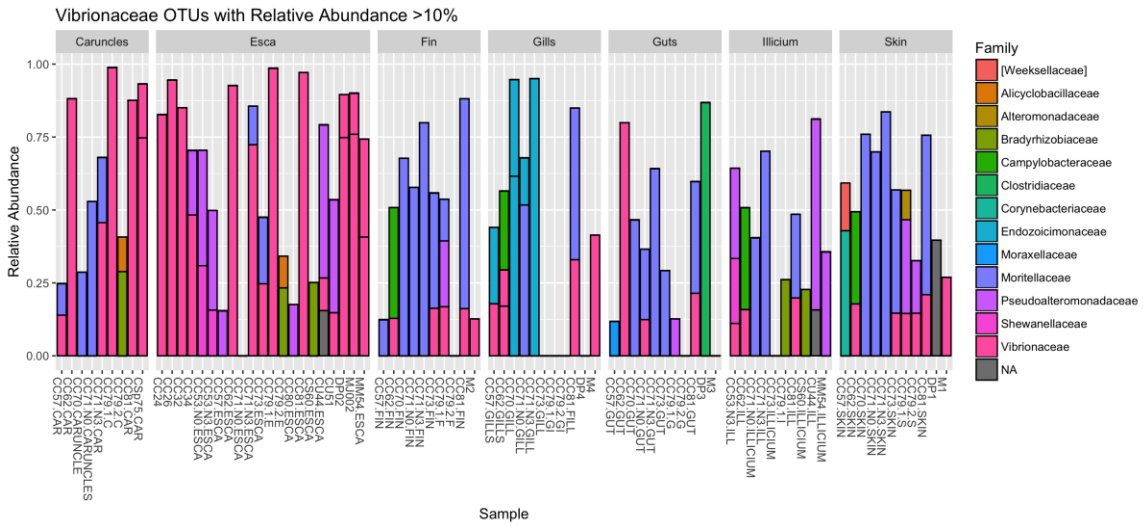


Figure 12. Bar plot of taxa present at greater than 10% relative abundance within adult anglerfish specimens by Family.

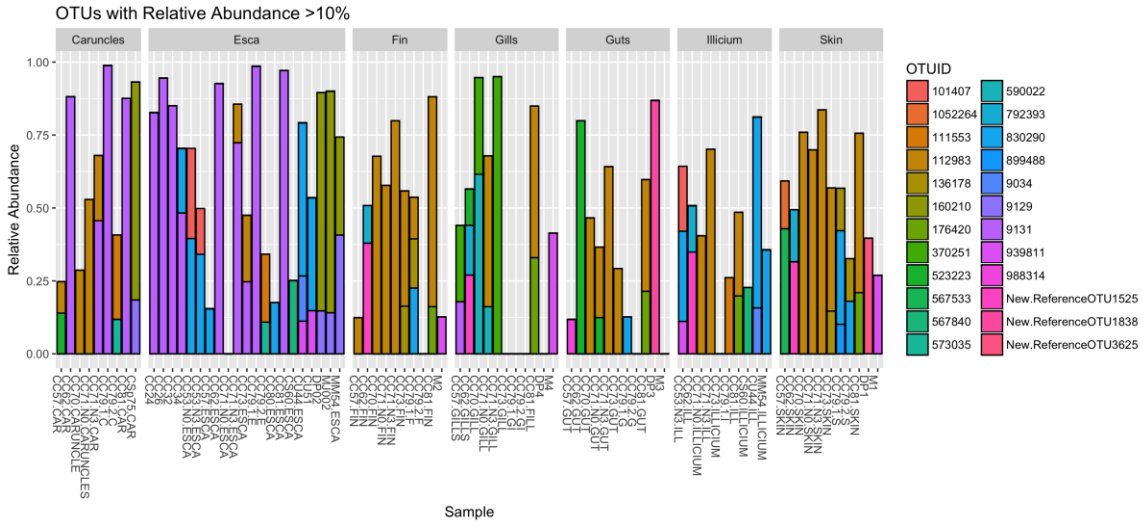


Figure 13. Bar plot of taxa present at greater than 10% relative abundance within adult anglerfish specimens by OTU ID.

Eight OTUs belonging to the family Vibrionaceae were present within anglerfish specimens at greater than 10% relative abundance (OTU IDs: 9131, 160210, 9129, 939811, 176420, 136178, 523223, and 792393). Of these, only five (9131, 160210, 9129, 939811, 523223) were found within the esca or caruncle of an adult anglerfish specimen (Figure 12).

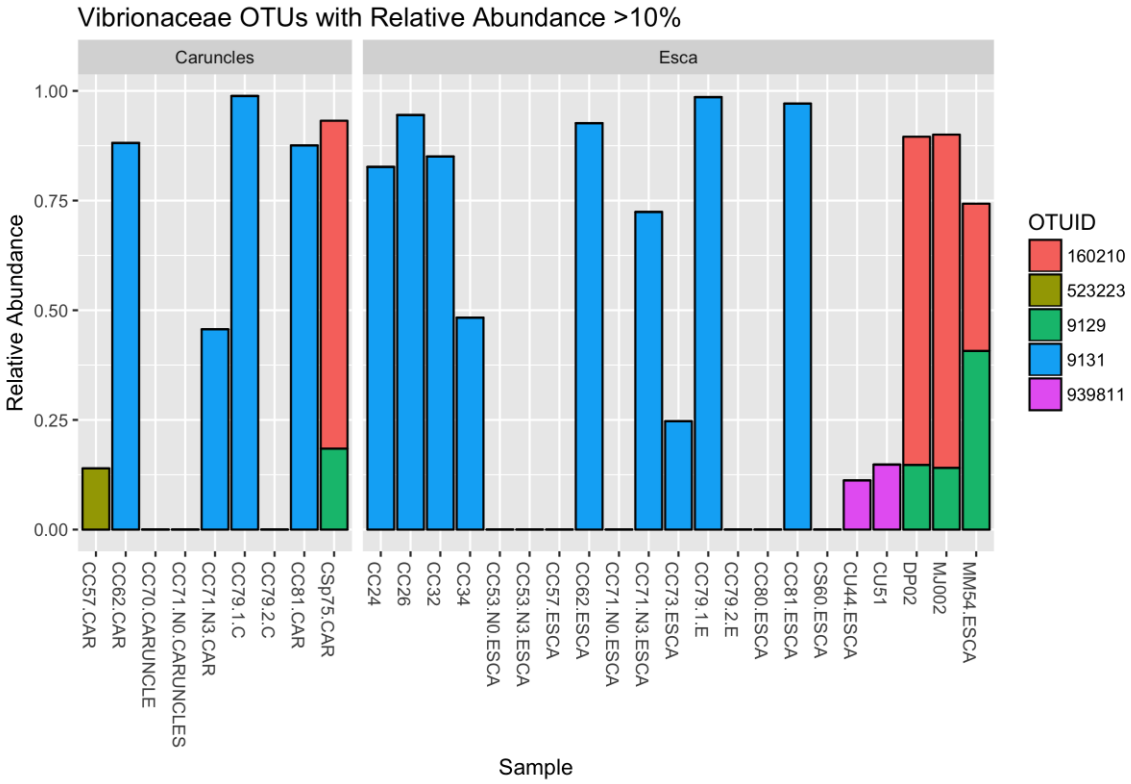


Figure 14. Bar plot of taxa belonging to family Vibrionaceae present at greater than 10% relative abundance within the bioluminescent organs of adult anglerfish specimens.

Sequencing revealed five potential bioluminescent symbiont taxa (OTU IDs: 9131, 160210, 9129, 523223, 939811). All taxa belonged to the family Vibrionaceae and accounted for greater than 10% of the relative abundance. OTUs 9129, 160210, and 939811 could only be identified to the family level as Vibrionaceae while OTU 9131 was placed within the genus *Enterovibrio*. OTU 523223 clustered at >97% identity to *Photobacterium angustum*. While most strains of *Photobacterium angustum* are not

known to exhibit bioluminescence, OTU 523223 was considered a potential bioluminescent symbiont as the luminous strain GB-1 had been provisionally included within the species (Urbanczyk *et al.*, 2010). These potential bioluminescent symbiont taxa may also be contaminants present on the external surface of the light organs.

OTU ID 9131 was identified with a relative abudance greater than 10% in nine escal specimens (all belonging to *C. couesii* hosts). While OTUs 9129 and 160210 were abundant within the escal specimens belonging to hosts within the families Melanocetidae and Oneirodidae. Within the escal specimens from both undefined *Ceratias* individuals OTU 939811 was the most abundant potential bioluminescent symbiont. No bioluminescent potential symbiont OTU was found at a relative abundance greater than 10% in seven of the 21 escal specimens.

OTU ID 9131 was identified within four of nine caruncle specimens with a relative abundance ranging from 45.6% - 98.8% (all *C. couesii* hosts). OTU IDs 9121 and 160210 were found within the caruncle specimens of an unknown host belonging to the genus *Ceratias*. Lastly, OTU 523223, which was not present in high abundance within the escal specimen of the same host nor within the escal specimens of other host species, was identified within the caruncle of a *C. couesii* host.

Of the seven *C. couesii* specimens from which an escal and caruncle sample were processed, five showed similar patterns of OTU abundance within both organ types. As stated above, individual CC57 contained OTU 523223 in an abundance greater than 10% within the caruncle but not within the esca. Specimens CC71.N0 and CC79.2 did not contain a high abundance of a potential bioluminescent symbiont OTU in either organ type.

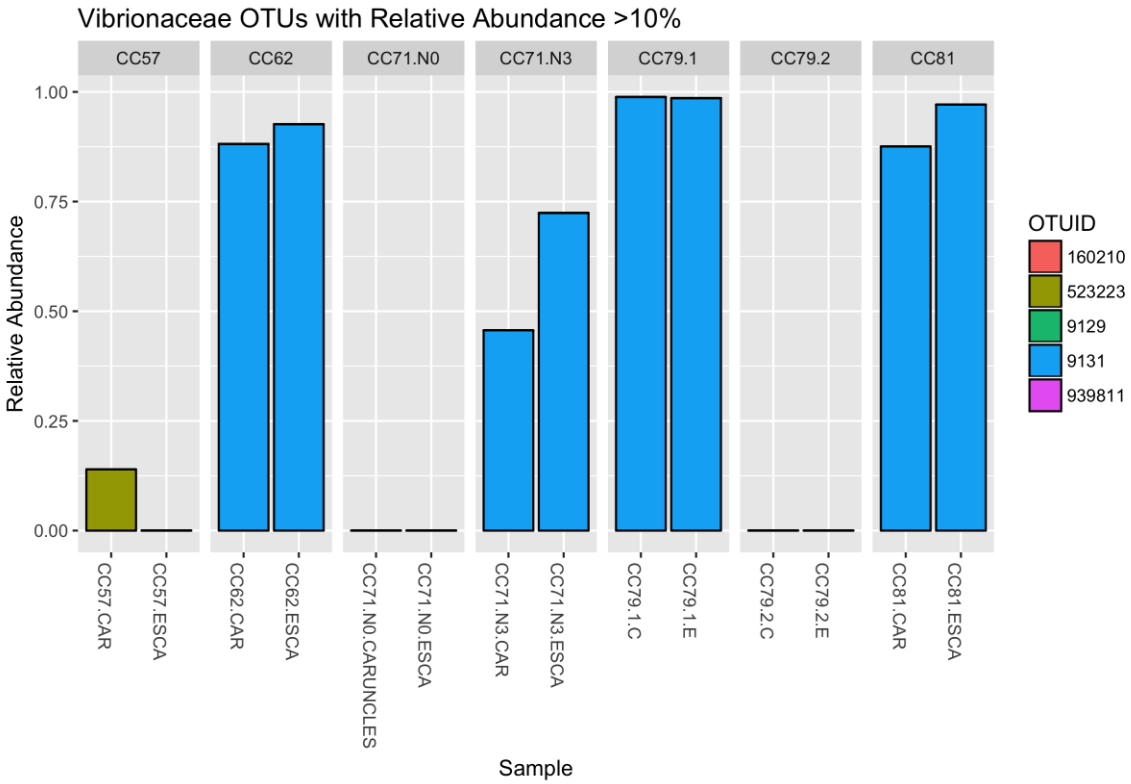


Figure 15. Relative abundance of symbiont OTUs corresponding to the family Vibrionaceae within caruncles and escae collected from the sample host individuals.

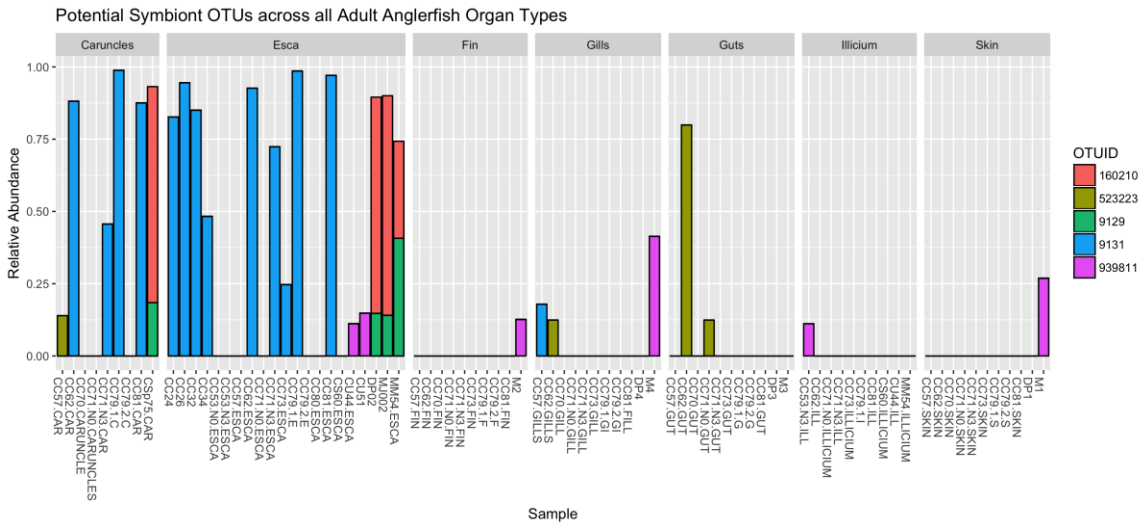


Figure 16. Relative abundance of potential symbiont OTUs from adult anglerfishes across all organ types of adult anglerfishes.

When examining the distribution of the five potential symbiont OTUs identified within the escal and caruncle specimens of adult anglerfishes across all organ types, OTUs 9131, 9129, and 160210 were mainly confined to the bioluminescent organs while 523223 and 939811 were present in several other organ types. This suggested that OTUs 9131, 9129, and 160210 were most likely to be bioluminescent symbionts cultured for the purpose of illuminating the esca and caruncles of their host. However, it is possible that bioluminescent symbionts could be cultured on the external surface of the fish or that these potential symbiont taxa were from the outer surface of the light organs.

Larval Anglerfish Symbiont Taxa

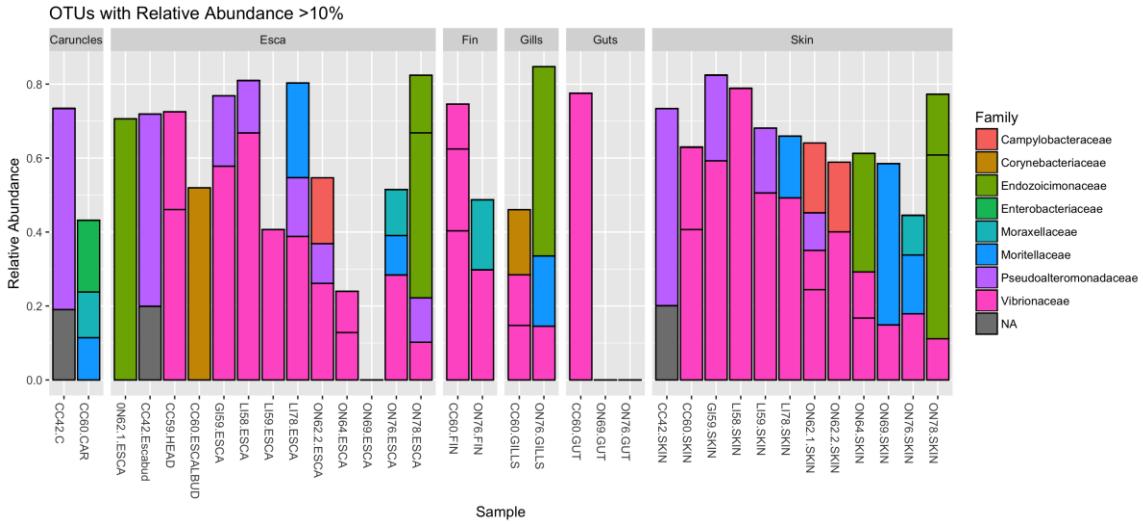


Figure 17. Bar plot of taxa present at greater than 10% relative abundance within larval anglerfish specimens, listed by Family.

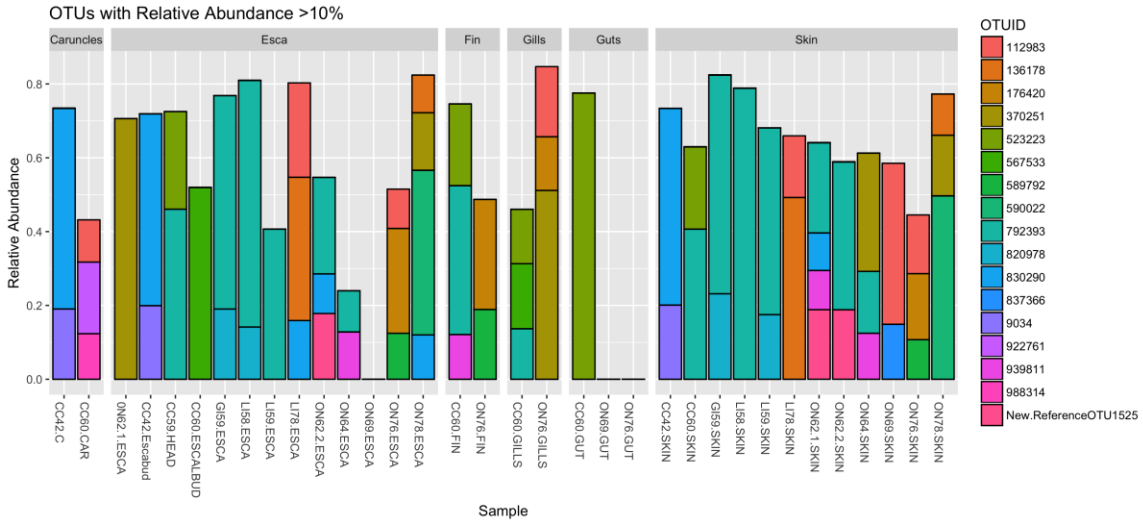


Figure 18. Bar plot of taxa present at greater than 10% relative abundance within larval anglerfish specimens, listed by OTU ID.

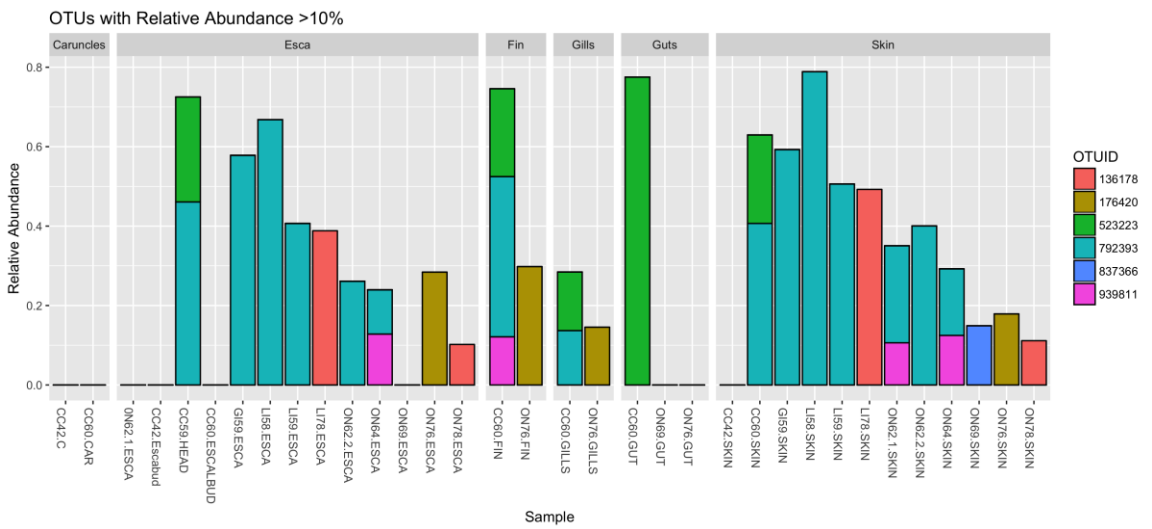


Figure 19. Bar plot of taxa belonging to family Vibrionaceae present at greater than 10% relative abundance within all organs of larval anglerfish specimens.

Larval anglerfish sequencing revealed six potential bioluminescent symbiont taxa (OTU IDs: 523223, 939811, 136178, 176420, 792393, 837366). All taxa belonged to the family Vibrionaceae and accounted for greater than 10% of the relative abundance within any organ type of a larval specimen. OTUs 523223 and 939811 were also identified within specimens from adult anglerfishes, but the other OTUs identified within larval specimens were not seen in high abundance within the adults. OTUs 136178, 176420 and 939811 could only be identified to the family level as Vibrionaceae while OTU 523223 and OTU 792393 clustered at >97% identity to *Photobacterium angustum* and *Vibrio shilonii,* respectively.

OTU ID 523223 was identified with a relative abudance greater than 10% in just one larval specimen which did not have a visible esca. OTU 136178 was present within the escal specimens of a larval Linophrynidae and a larval Oneirodidae specimen. OTU 176420 was present in high abundance within only one specimen, an esca from a Linophrynidae larva. 939811 was also present in only one specimen, an esca from an Oneirodidae larva. Lastly, OTU 792393 was the most abundant across all larval escal specimens with a relative abundance ranging from 11.1% to 66.8% across six of the 13 samples.

While none of the three most likely OTUs identified as potential bioluminescent symbionts (9131, 9129, and 160210) within the adult anglerfish specimens were present with a relative abundance level greater than 10% in the larval specimens, they were present at very low levels (Supplemental Table 12).

Unlike the adult specimens, the potential symbiont OTUs identified within the escal and caruncle specimens of larval anglerfishes were also present at fairly high abundance within the other organ types. Without a paired adult for comparison, it was not possible to determine whether the symbiont OTUs identified in the larval specimens were most likely to be cultured by the host for the purpose of illuminating the esca and caruncles.

Anglerfish Symbiont Taxa in Seawater

All eight potential symbiont OTUs were detected in at least 41 of the 214 seawater samples at low relative abundance levels ranging from 0 - 0.66% per sample. OTU 523223 was most abundant across all seawater samples followed by OTUs 939811, 9131, 176420, 837366, 136178, 160210, and 9121 respectively. However, when examined by depth, symbiont OTUs were on average most abundant within the mesopelagic and bathypelagic zones (Figure 19, Figure 20, Supplemental Table 13).

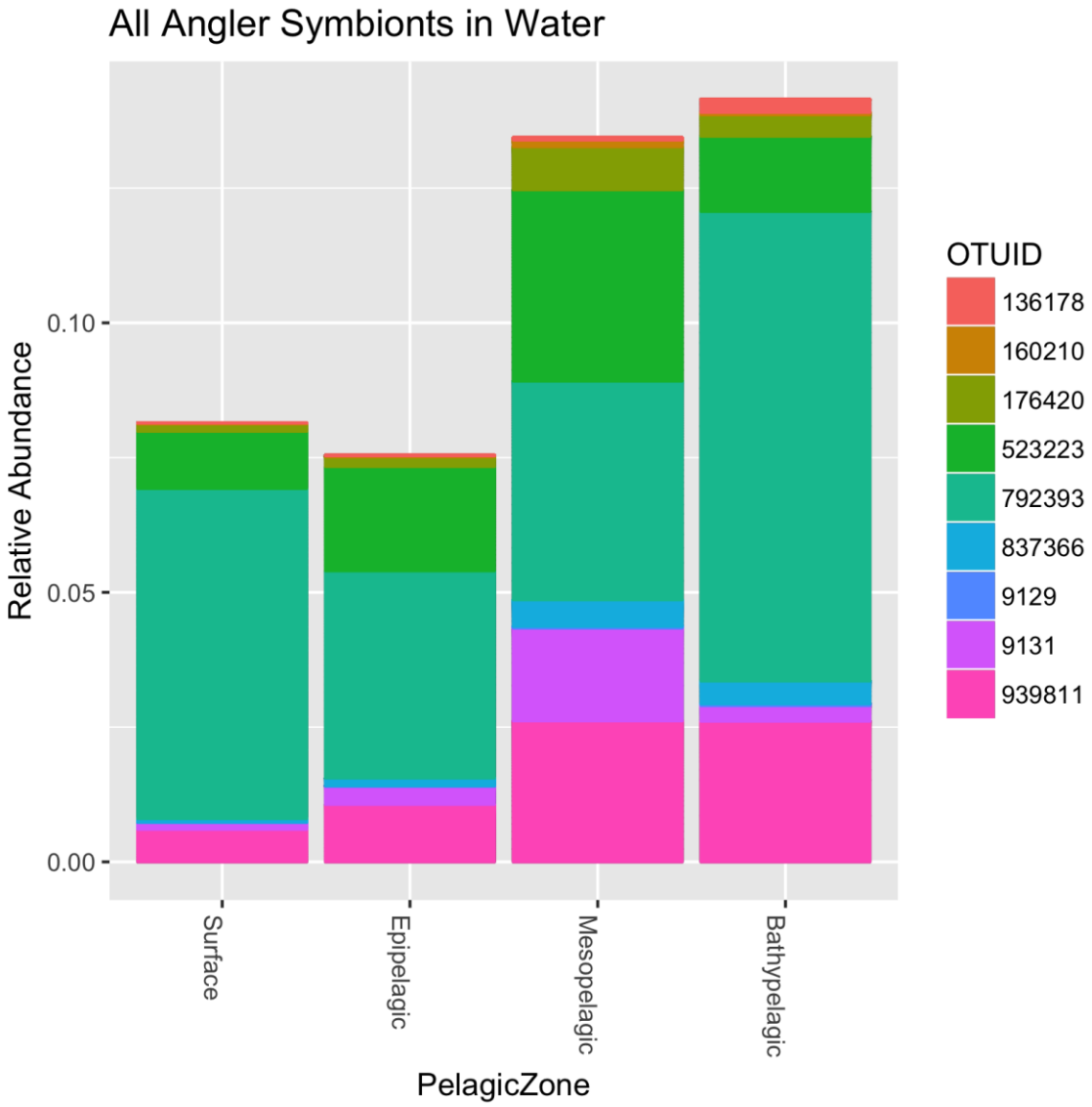


Figure 20 Bar plot of relative abundance of all potential symbiont OTUs by depth zone

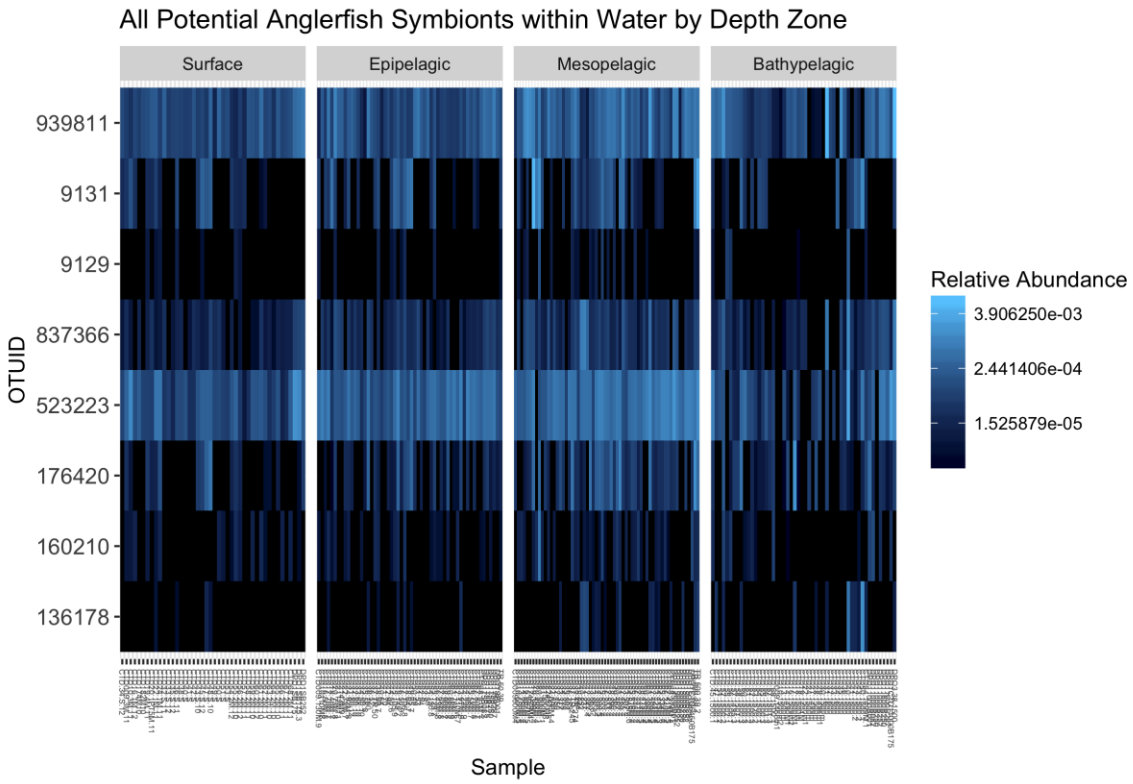


Figure 21. Heatmap of relative abundance of all potential symbiont OTUs by depth zone.

DISCUSSION

Microbiomes of Anglerfish and the Environment

Not unlike the findings of prior studies on fish-associated microbiomes and their environment (Larsen *et al.*, 2015; Legrand *et al.*, 2018; Pratte *et al.*, 2018), there existed a significant difference in the richness and diversity of the microbial community found within all tested organ types of the anglerfish specimens and the surrounding environment (Figure 3). The greatest difference between the two was the greater abundance of the genera *Moritella*, *Pseudoalteromonas*, *Enterovibrio*, and *Vibrio* within anglerfish specimens as compared to the water.

OTU 112983 represented an unknown species within the genus *Moritella* and was present at high abundance levels within all organs of adult anglerfishes. Members of the genus *Moritella* are generally piezophilic and are suspected to form mutualistic relationships with deep-sea organisms (Urakawa, 2013). One member of the genus, *M. viscosa*, is known to cause skin ulcerations in fish (Urakawa, 2013). Also present at high

abundance levels within the escae and illicia of adult anglerfishes was OTU 830290 representing the genus *Pseudoalteromonas.* Known members of *Pseudoalteromonas* have been reported to provide antifouling and/or algicidal benefits (Holmström and Kjelleberg, 1999). This genus also appears to be one of the more culturable marine bacteria (Sfanos *et al.*, 2005). More detailed investigation may be beneficial to determine if the taxa identified here also exhibit antifouling properties which may in turn aid the host in reducing the presence of microbes that compete with or prevent colonization by bioluminescent symbionts. Lastly, the genera *Enterovibrio* and *Vibrio* are typically host-associated and both contain luminous species (Dunlap and Urbanczyk, 2013; Hendry *et al.*, 2018).

Microbial Communities – Adult Anglerfish

Examining adult anglerfish specimens by organ type did not reveal any significant differences in regards to microbial richness or diversity. However, the escae and caruncles of adult anglerfishes had the lowest levels of microbial richness and diversity in comparison to other organ types sampled. The lack of significant difference may be in part due to the fact that the entire bioluminescent organ was processed, including the epithelial surface; including the outer skin of the organ in the extraction process may have inflated the diversity and richness of these organs.

Bray-Curtis dissimilarity analysis revealed that the collection site (station) accounted for the greatest percentage of variation seen within adult anglerfish specimens. This was primarily driven by the high abundance of *Moritella* sp. present in samples collected from stations SW5 and B175. Nevertheless, samples were unevenly sampled across stations, so it is difficult to draw any strong conclusions. Host species accounts for the second greatest percentage of variation seen within adult anglerfish microbial communities. Several previous studies have indicated that host species plays a significant role in the microbial communities of fish (Larsen *et al.*, 2013; Boutin *et al.*, 2014; Pratte *et al.*, 2018). These findings indicate that the microbiome of adult anglerfishes may be influenced in part by the environment but may also be regulated by host specific relationships with microbes.

Microbial Communities – Larval Anglerfish

Like adults, collection location (station) explained the greatest percentage of variation within the microbial communities of larval anglerfishes. However, collection depth was the second strongest driver of beta diversity. Unfortunately due to the nature of sample collection, a large portion of larval specimens were collected from net N0, which collected samples throughout the entire descent from the surface to the maximum depth of 1500 m, so we were unable to discern at which discrete depth the specimen was collected. These samples were binned together and thereby reduce the strength of this observation.

Adult Anglerfish Bioluminescent Symbionts

The bioluminescent organs of adult anglerfishes were dominated by OTUs 9131, 160210, and 9129, with OTUs 523223 and 939811 also present, but less distinct. These results indicated a potential host-species specific symbiotic relationship between *C. couesii* host and symbiont OTU 9131. This is supported by previous 16S sequencing as well as current full genome sequencing of the *C. couesii* bioluminescent symbiont (Haygood *et al.*, 1992; Hendry *et al.*, 2018).

However, symbiont analysis also indicated the possibility of dual symbionts within the bioluminescent organs of two Melanocetidae, one *Dolopichthys*, and an unknown *Ceratias* host. Where present, OTUs 160210 and 9129 appear together in high abundance. Previous study of the *M. johnsonii* symbiont matches to OTU 9129 and current full genome sequencing of the *M. johnsonii* bioluminescent symbiont indicates a single symbiont species (Hendry *et al.*, 2018). In addition, the reference sequences for these two OTUs differed by only seven basepairs (97% identical). Therefore, OTU 160210 may be a remnant of the OTU picking process and not necessarily a secondary symbiont taxon.

OTU 523223 was found in high abundance within the caruncle of a single *C. couesii* specimen while OTU 939811 was identified within the escae of specimens of an undescribed *Ceratias* species (Sutton et al., in prep.). However, these potential symbiont OTUs were present at fairly high abundance levels within other organ types. It is unclear from this analysis whether these OTUs were indeed bioluminescent symbionts cultured

for the purpose of illuminating the anglerfishes' escae. Future full genome sequencing may help to shed light on the likelihood that these taxa represent a bioluminescent symbiont.

For the *C. couesii* specimens for which a caruncle and escal specimen were collected, when one of the identified potential symbiont OTUs was present, it was found in high abundance within both organ types. This confirms prior observations of bioluminescent bacteria oozing from the caruncles of freshy collected specimens (Pietsch, 2009) and indicates that the same symbiont taxa are cultivated by the host in both luminous organs. It has also been hypothesized that the illicium may provide a way for the bioluminescent symbiont to be transferred from the caruncle to the esca (Pietsch, 2009), but OTU 9131 was not identified at high abundance levels within the illicia of adult *C. couesii* individuals. Since the *C. couesii* symbiont (OTU 9131) was not detected at >10% relative abundance within the illicium of any *C. couesii* individual for which an escal and caruncle specimen was also processed, it was concluded that the illicium does not provide a continuous means for symbiont transport between the caruncle and esca of adult *C. couesii*.

Larval Anglerfish Bioluminescent Symbionts

Without an adult specimen of the same species with which to compare, we cannot draw many strong conclusions regarding bioluminescent symbionts within larvae, but it is worth noting that OTU 9131, which was found in high abundance within adult *C. couesii* anglerfishes, was identified at lower relative abundance levels (0.01-0.11%) within the primordial escae and caruncles of the three larval *C. couesii* specimens. The presence of the symbiont OTU could indicate that the larvae may have been inoculated by their mother (Pietsch, 2009). However, the relative abundance level of OTU 9131within *C. couesii* larval specimens was not dramatically greater than the relative abundance of OTU 9131 within seawater samples (0 – 0.66%). Without a more controlled comparison, it is difficult to definitively conclude that the symbiont detected within the larval samples is due to either vertical transmission or environmental acquisition. It should also be noted that these larvae were collected at depths between 10 m and 999 m so it is possible that the larvae had already begun their ontogenetic vertical migration.

Based on taxonomic assignment, although most abundant in larval specimens, OTU 792393 is not likely to be a bioluminescent symbiont as *Vibrio shilonii* does not luminesce (Kushmaro *et al.*, 2001).The remaining potential symbiont OTUs identified at high abundance in the escal specimens of larvae (523223, 939811, 136178, 176420, 837366) were also found at abundance levels >10% in at least one other organ type. This may be an indication that the bioluminescent symbionts are not limited solely to the escal region and may grow on the body of larval anglerfishes, or that non-symbiotic members of the Vibrionaceae family are also present at high abundance levels on larvae. Full genome sequencing of potential larval symbionts as well as additional sampling and analysis of corresponding adults would aid in clarifying this observation.

Bioluminescent Symbionts within Seawater

In order to examine the possibility that the larvae may be acquiring symbionts from their environment, we searched for the potential symbionts within seawater samples. Traces of all eight potential symbionts were found within the water at very low levels of relative abundance. This finding may imply that the bioluminescent symbionts of ceratioids are not obligately dependent, as they are able to survive outside of the host and therefore are more likely to be acquired from the environment as is seen in other symbiotic relationships between bioluminescent bacteria and fishes (Dunlap and Urbanczyk, 2013). These findings are also supported by the recent full genome analysis of the *C. couesii* bioluminescent symbiont, which indicated that the symbiont has retained motility genes required for development of a flagellum (Hendry *et al.*, 2018). In addition, all eight potential symbionts were found at the greatest abundance within the mesopelagic and bathypelagic zones. A greater concentration of these OTUs at depth also supports the hypothesis that larval anglerfishes acquire bioluminescent symbionts from the environment as the esca develops and the larvae make their ontogenetic migration from the surface waters to the bathypelagic zone (Pietsch, 2009).

Symbiont Transmission

Based on the results of this study, a clear and simple pattern of symbiont transmission was not observed. There appears to be some host-specificity as seen between OTU 9131

and adult *C. couesii*, but this relationship was not seen in the limited number of conspecific larvae sampled. In addition, the detection of symbiont OTUs within seawater suggests that environmental acquistion is a plausible mode of symbiont transmission. While neither vertical transmission nor horizontal acquisition alone explain these observations, these two modes of transmission are not necessarily mutually exclusive (Bright and Bulgheresi, 2010). There have been described many intermediate modes of symbiont transmission, which may provide a more plausible explanation for the observed relationship between ceratioids and their bioluminescent symbionts (Wilkinson and Sherratt, 2001; Bright and Bulgheresi, 2010).

While our results suggest larval ceratioids are most likely to encounter free-living bioluminescent symbionts as they make their ontogenetic vertical migration to the mesopelagic and bathypelagic zones, it is possible that the symbiont OTUs detected within the seawater are a result of the release of bioluminescent bacteria by adult anglerfishes (Bright and Bulgheresi, 2010; Hendry *et al.*, 2016). Deep-sea anglerfishes may be using a combination of transmission methods, such as pseudo-vertical transmission. While larvae may not be acquiring symbionts directly from their mothers, it is still possible that they are acquiring symbionts from a parent generation. Such a mode of transmisson would support the host-specificity observed for *C. couesii*, but can also creates an opportunity for "partner-choice" which may explain the lack of specificity observed across other ceratioid host families (Wilkinson and Sherratt, 2001). While some mystery still surrounds the relationship between deep-sea anglerfishes and their bioluminescent symbionts, molecular advances allow us to investigate and explore the countless ways that bacteria can interact with and affect animals (McFall-Ngai *et al.*, 2013).

CONCLUSION

This study provides new insights into the microbial communities associated with deep-sea ceratioids. Our findings support the previous identification of differing bioluminescent symbionts within *C. couesii* and *M. johnsonii* host specimens, but also indicate that bioluminescent symbionts may not be specific at the host family level. The microbiomes of adult ceratioids contained greater abundance of OTUs representing taxa of the *Moritella* and *Pseudoalteromonas* genera when compared to seawater samples. We hypothesize that these taxa may assist in symbiont acquisition by reducing competition for colonization of the light organs. Adult bioluminescent symbiont OTUs were not found in high abundance within larval ceratioids, however additional Vibrionaceae OTUs were identified at >10% relative abundance. Future sequencing studies would be beneficial in determining whether these OTUs represent luminous species. Lastly, the identification of OTUs representing the bioluminescent symbionts within seawater provides evidence that the ceratioid bioluminescent symbionts are not obligately dependent upon the host for growth. All of these findings provide support for the hypothesis that ceratioids acquire their bioluminescent symbionts from the environment as larvae metamorphose and make their ontogenetic migration to the bathypelagic.

APPENDIX 1
Supplemental Tables

Supplemental Table 1. Sequencing Statistics.

	All Samples	Anglerfish Only	Water Only	All Samples with Water Merged
Total # of samples	734	117	617	331
Total # of reads	64,145,146	6,876,285	57,268,861	64,145,146
Mean # of reads	87,391	58,771	92,818	193,792

Supplemental Table 2 SIMPER analysis comparing all anglerfish to water sample OTUs, up to a cumulative sum of .5 (50.0%).

Contrast: Anglerfish_Water							
OTU ID	average	sd	ratio	ava	avb	cumsum	p
112983	0.148195	0.20909	0.7088	1.355e-01	3.551e-04	0.1549	0.002**
830290	0.091257	0.15006	0.6081	7.703e-02	1.045e-02	0.2503	0.002**
9131	0.083789	0.20829	0.4023	9.514e-02	9.346e-05	0.3379	0.002**
792393	0.064024	0.15137	0.4230	6.150e-02	1.140e-03	0.4048	0.002**
355538	0.046191	0.07536	0.6129	1.707e-03	3.802e-02	0.4531	1.000
823476	0.041509	0.07681	0.5404	7.086e-03	3.369e-02	0.4965	1.000

Significant codes: *=.05, **=.01

Supplemental Table 3. Mean alpha diversity measurements for adult anglerfish by sample type.

Sample Type	Observed	Chao1	Shannon	InvSimpson
Caruncles	62.88889	91.47158	1.890817	5.305612
Esca	74.50000	135.57745	1.988521	7.263346
Fin	140.72727	295.73510	2.799701	13.704170
Gills	122.09091	276.31860	2.650666	13.408331
Guts	168.58333	326.98766	3.297943	44.625079
Illicium	107.40000	188.19745	2.881687	9.829678
Skin	140.50000	342.30563	2.626287	6.403103

Supplemental Table 4. Tukey HSD results for Sample Types by diversity index.

	P adj			
	Observed	Chao1	Shannon	InvSimpson
Esca-Caruncles	0.9999984	0.9999551	0.9999906	1.0000000
Fin-Caruncles	0.4989612	0.7702222	0.3658370	0.9995015
Gills-Caruncles	0.7818795	0.8427090	0.4671511	0.9993423
Guts-Caruncles	0.1217373	0.5975663	0.0061799*	0.1219671
Illicium-Caruncles	0.9545514	0.9963275	0.2260845	0.9999917
Skin-Caruncles	0.8845868	0.8544492	0.9396425	1.0000000
Water-Caruncles	0.0000000***	0.0000000***	0.0000000***	0.0000407***
Fin-Esca	0.3404251	0.7707209	0.2409247	0.9995590
Gills-Esca	0.6956293	0.8585862	0.3468124	0.9993745
Guts-Esca	0.0324866	0.5361084	0.0004420***	0.0237947**
Illicium-Esca	0.9502294	0.9995555	0.1326850	0.9999987
Skin-Esca	0.7951302	0.8411035	0.9279721	0.9999990
Water-Esca	0.0000000***	0.0000000***	0.0000000***	0.0000000***
Gills-Fin	0.9998511	0.9999999	0.9999999	1.0000000
Guts-Fin	0.9973966	0.9999989	0.8217374	0.3432213
Illicium-Fin	0.9932650	0.9935232	0.9999603	0.9999987
Skin-Fin	0.9815822	0.9999363	0.8780540	0.9980981
Water-Fin	0.0000000***	0.0000015***	0.0000000***	0.0004499**
Guts-Gills	0.9472836	0.9999620	0.7262615	0.3577210
Illicium-Gills	0.9999502	0.9979408	0.9995772	0.9999975
Skin-Gills	0.9998605	0.9999983	0.9405403	0.9975428
Water-Gills	0.0000000***	0.0000005***	0.0000000***	0.0005151**
Illicium-Guts	0.8219655	0.9707076	0.9706257	0.2769928
Skin-Guts	0.6322104	0.9979102	0.0399104	0.0269586**
Water-Guts	0.0000000***	0.0000008***	0.0000000***	0.7902431
Skin-Illicium	1.0000000	0.9995075	0.6997636	0.9999615
Water-Illicium	0.0000000***	0.0000001***	0.0000000***	0.0006041**
Water-Skin	0.0000000***	0.0000000***	0.0000000***	0.0000000***

Supplemental Table 5. Tukey HSD results for Inverse Simpson diversity index by Larval Sample Type.

	P adj
	InvSimpson
Esca-Caruncles	1.0000000
Fin-Caruncles	0.9999661
Gills-Caruncles	1.0000000
Guts-Caruncles	0.0684236
Skin-Caruncles	0.9992507
Fin-Esca	0.9999361
Gills-Esca	1.0000000
Guts-Esca	0.0031058
Skin-Esca	0.9901263
Gills-Fin	0.9999686
Guts-Fin	0.0436800
Skin-Fin	0.9999979
Guts-Gills	0.0679755
Skin-Gills	0.9992879
Skin-Guts	0.0012728

Supplemental Table 6. Taxa of OTU IDs present in caruncles and escal specimens of adult anglerfish samples with relative abundance >10% per GreenGenes reference sequence taxa assignment.

OTUID	Class	Order	Family	Genus	Species
101407	Gammaproteobacteria	Alteromonadales	Shewanellaceae	*Shewanella*	*NA*
112983	Gammaproteobacteria	Alteromonadales	Moritellaceae	*Moritella*	*NA*
9034	Gammaproteobacteria	Alteromonadales	*NA*	*NA*	*NA*
9131	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Enterovibrio*	*NA*
9129	Gammaproteobacteria	Vibrionales	Vibrionaceae	*NA*	*NA*
160210	Gammaproteobacteria	Vibrionales	Vibrionaceae	*NA*	*NA*
523223	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Photobacterium*	angustum
939811	Gammaproteobacteria	Vibrionales	Vibrionaceae	*NA*	*NA*
573035	Bacilli	Bacillales	Alicyclobacillaceae	*Alicyclobacillus*	*NA*
111553	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	*NA*
567840	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*NA*	*NA*
830290	Gammaproteobacteria	Vibrionales	Pseudoalteromonadaceae	*Pseudoalteromona*	*NA*

Supplemental Table 7. Relative abundance of symbiont OTUs within escal specimens from adult anglerfishes by host taxa.

Host taxa	Escal Specimen ID	OTU ID				
		9131	9129	160210	523223	939811
Cryptopsaras couesii	CC24	0.8268	0.0000	0.0000	0.0000	0.0066
	CC26	0.9452	0.0000	0.0000	0.0000	0.0005
	CC32	0.8505	0.0000	0.0001	0.0000	0.0074
	CC34	0.4830	0.0000	0.0002	0.0000	0.0081
	CC53.N0.ESCA	0.0001	0.0000	0.0000	0.0018	0.0515
	CC53.N3.ESCA	0.0963	0.0000	0.0001	0.0008	0.0884
	CC57.ESCA	0.0736	0.0000	0.0000	0.0239	0.0001
	CC62.ESCA	0.9265	0.0000	0.0003	0.0013	0.0030
	CC71.N0.ESCA	0.0439	0.0000	0.0000	0.0000	0.0000
	CC71.N3.ESCA	0.7239	0.0000	0.0003	0.0000	0.0018
	CC73.ESCA	0.2471	0.0000	0.0000	0.0000	0.0001
	CC79.1.E	0.9858	0.0000	0.0000	0.0000	0.0000
	CC79.2.E	0.0000	0.0000	0.0000	0.0000	0.0000
	CC80.ESCA	0.0407	0.0000	0.0000	0.0100	0.0081
	CC81.ESCA	0.9709	0.0001	0.0003	0.0000	0.0007
Centrophryne spinulosa	CS60.ESCA	0.0076	0.0000	0.0000	0.0114	0.0087
Undescribed *Ceratias* sp.	CU44.ESCA	0.0000	0.0129	0.0116	0.0000	0.1119
	CU51	0.0001	0.0096	0.0329	0.0001	0.1481
Dolopichthys sp.	DP02	0.0010	0.1475	0.7480	0.0001	0.0019
Melanocetus johnsonii	MJ002	0.0002	0.1408	0.7595	0.0000	0.0394
Melanocetus murrayi	MM54.ESCA	0.0004	0.4074	0.3355	0.0001	0.0001

Supplemental Table 8. Relative abundance of symbiont OTUs within caruncle specimens from adult anglerfishes by host taxa.

Host Taxa	Caruncle Specimen ID	OTU ID				
		9131	9129	160210	523223	939811
Cryptopsaras couesii	CC57	0.0147	0.0000	0.0001	0.1396	0.0009
	CC62	0.8815	0.0000	0.0002	0.0033	0.0055
	CC70	0.0013	0.0000	0.0000	0.0000	0.0001
	CC71.N0	0.0491	0.0000	0.0000	0.0000	0.0000
	CC71.N3	0.4564	0.0001	0.0002	0.0002	0.0028
	CC79.1	0.9883	0.0000	0.0000	0.0000	0.0000
	CC79.2	0.0000	0.0000	0.0000	0.0031	0.0000
	CC81	0.8755	0.0000	0.0001	0.0000	0.0014
Ceratias sp.	CSp75	0.0003	0.1846	0.7474	0.0000	0.0011

Supplemental Table 9. Taxa of OTUID present in caruncles and escal specimens of larval anglerfish samples with relative abundance >10% per GreenGenes reference sequence taxa assignment

OTUID	Order	Family	Genus	Species
112983	Alteromonadales	Moritellaceae	*Moritella*	NA
9034	Alteromonadales	NA	NA	NA
523223	Vibrionales	Vibrionaceae	*Photobacterium*	*angustum*
136178	Vibrionales	Vibrionaceae	NA	NA
176420	Vibrionales	Vibrionaceae	NA	NA
837366	Vibrionales	Vibrionaceae	NA	NA
939811	Vibrionales	Vibrionaceae	NA	NA
820978	Vibrionales	Pseudoalteromonadaceae	NA	NA
792393	Vibrionales	Vibrionaceae	*Vibrio*	*shilonii*
922761	Enterobacteriales	Enterobacteriaceae	NA	NA
567533	Actinomycetales	Corynebacteriaceae	*Corynebacterium*	NA
590022	Oceanospirillales	Endozoicimonaceae	NA	NA
370251	Oceanospirillales	Endozoicimonaceae	NA	NA
589792	Pseudomonadales	Moraxellaceae	*Psychrobacter*	*pacificensis*
988314	Pseudomonadales	Moraxellaceae	*Acinetobacter*	NA
New.ReferenceOTU1525	Campylobacterales	Campylobacteraceae	*Arcobacter*	NA
830290	Vibrionales	Pseudoalteromonadaceae	*Pseudoalteromonas*	NA

Supplemental Table 10. Relative abundace of potenial larval symbionts within escal specimens by host taxa.

Host Taxa	Escal Specimens	OTU ID				
		523223	136178	176420	939811	792393
Cryptopsaras couesii	CC42.Escabud	0.0008	0.0000	0.0021	0.0593	0.0069
	CC59.HEAD	0.2643	0.0000	0.0020	0.0409	0.4608
	CC60.ESCALBUD	0.0233	0.0000	0.0000	0.0008	0.0057
Gigantactinidae	GI59.ESCA	0.0232	0.0000	0.0003	0.0272	0.5782
Linophrynidae unknown	LI58.ESCA	0.0074	0.0000	0.0022	0.0319	0.6682
	LI59.ESCA	0.0306	0.0007	0.0380	0.0247	0.4066
	LI78.ESCA	0.0005	0.3882	0.0010	0.0001	0.0000
Oneirodidae unknown	0N62.1.ESCA	0.0022	0.0000	0.0000	0.0311	0.0834
	ON62.2.ESCA	0.0208	0.0000	0.0037	0.0796	0.2613
	ON64.ESCA	0.0112	0.0077	0.0381	0.1283	0.1114
	ON69.ESCA	0.0093	0.0472	0.0000	0.0000	0.0000
	ON76.ESCA	0.0049	0.0022	0.2841	0.0307	0.0000
	ON78.ESCA	0.0000	0.1020	0.0003	0.0000	0.0000

Supplemental Table 11. Relative abundance of potential larval symbionts within caruncle specimens by host taxa.

Host Taxa	Caruncle Specimens	OTU ID				
		523223	136178	176420	939811	792393
Cryptopsaras couesii	CC42.C	0.0006	0.0001	0.0029	0.0461	0.0057
	CC60.CAR	0.0486	0.0000	0.0000	0.0001	0.0001

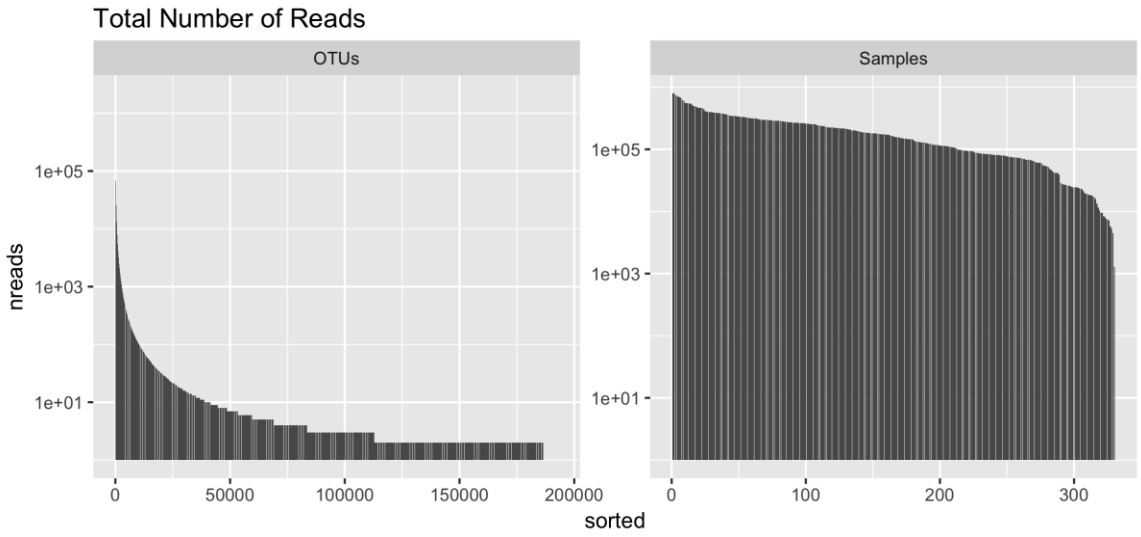
Supplemental Table 12. Presence of potential symbiont OTUs identified in adult specimens within larvae escal and caruncle specimens.

	9131	9129	160210	523223	939811
0N62.1.ESCA	0.0002	0.0000	0.0000	0.0022	0.0311
CC42.C	0.0011	0.0002	0.0001	0.0006	0.0461
CC42.Escabud	0.0002	0.0000	0.0000	0.0008	0.0593
CC59.HEAD	0.0010	0.0000	0.0001	0.2643	0.0409
CC60.CAR	0.0001	0.0001	0.0000	0.0486	0.0001
CC60.ESCALBUD	0.0001	0.0001	0.0000	0.0486	0.0001
GI59.ESCA	0.0001	0.0001	0.0000	0.0486	0.0001
LI58.ESCA	0.0001	0.0001	0.0000	0.0486	0.0001
LI59.ESCA	0.0001	0.0001	0.0000	0.0486	0.0001
LI78.ESCA	0.0001	0.0001	0.0000	0.0486	0.0001
ON62.2.ESCA	0.0001	0.0001	0.0000	0.0486	0.0001
ON64.ESCA	0.0001	0.0001	0.0000	0.0486	0.0001
ON69.ESCA	0.0001	0.0001	0.0000	0.0486	0.0001
ON76.ESCA	0.0001	0.0001	0.0000	0.0486	0.0001
ON78.ESCA	0.0001	0.0001	0.0000	0.0486	0.0001

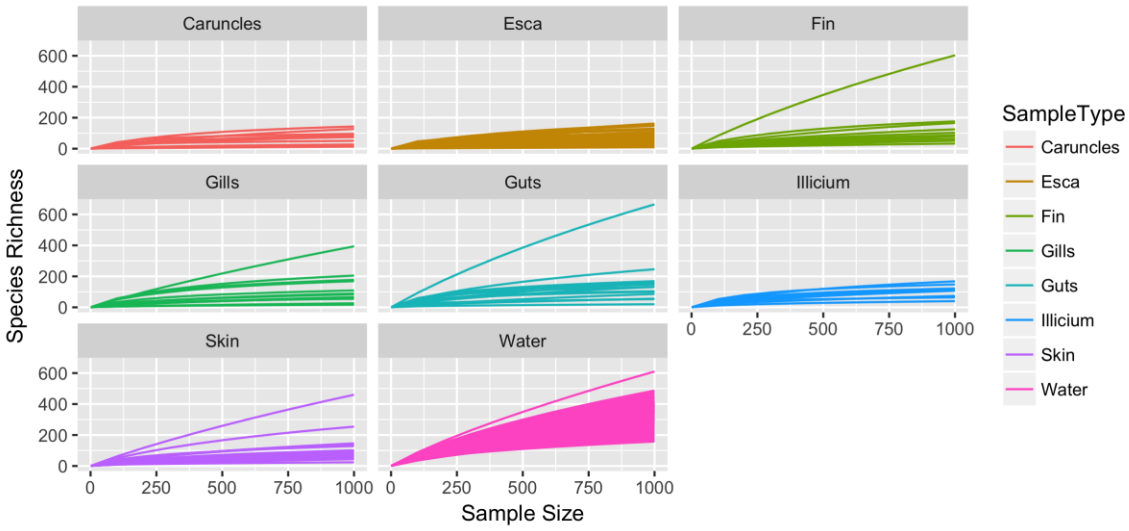
Supplemental Table 13. Mean relative abundance of all potential symbiont OTUs by depth.

	9131	9129	160210	523223	136178	176420	837366	939811	792393
Surface	2.87E-05	1.14E-06	3.57E-06	2.41E-04	6.00E-07	3.10E-05	1.55E-05	1.37E-04	1.39E-03
Epipelagic	6.01E-05	1.50E-06	3.76E-06	3.46E-04	9.75E-07	3.23E-05	2.59E-05	1.90E-04	6.86E-04
Mesopelagic	2.78E-04	5.39E-06	1.98E-05	5.72E-04	7.05E-06	1.29E-04	7.93E-05	4.22E-04	6.54E-04
Bathypelagic	5.57E-05	6.68E-06	8.56E-06	2.68E-04	4.63E-05	7.64E-05	8.13E-05	5.02E-04	1.68E-03

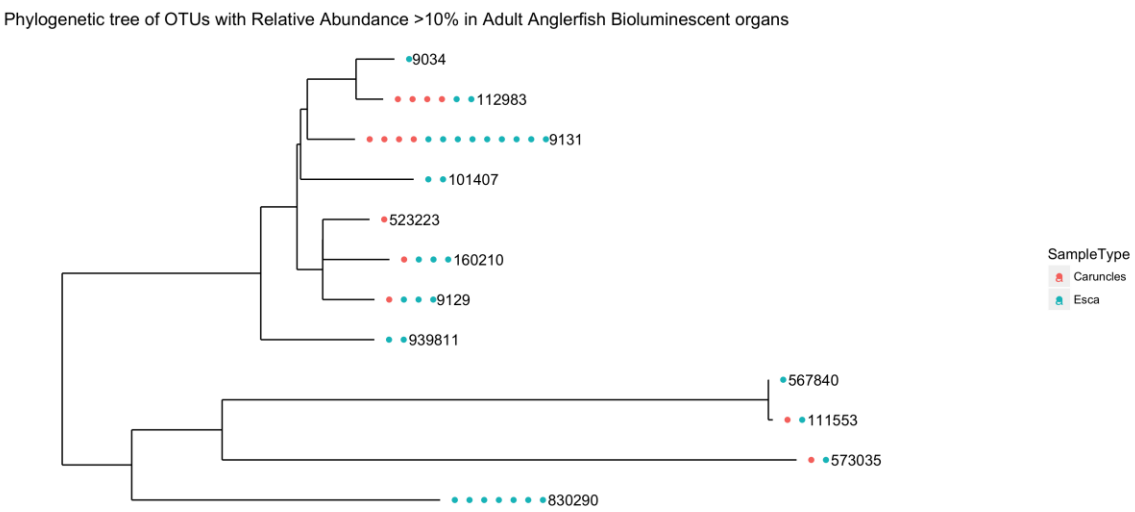
Supplemental Figures



Supplemental Figure 1. Total number of reads per OTU and per Sample.



Supplemental Figure 2. Rarefaction curve for all samples following rarefication to 1000 reads per sample.



Supplemental Figure 3. Phylogenetic tree of OTUs with a relative abundance >10% in adult anglerfish bioluminescent organs.

Supplemental R Code

Supplemental R code 1. Results for Adonis pair-wise comparisons of all variables. Parameters include: anglerfish organ type (SampleType), collection station (Station), collection depth range (PelagicZone), anglerfish taxonomic family (Angler.Family), and anglerfish species (Angler.Taxa).

```
> adonis.SampleType

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ SampleType,      data = as(samp
le_data(ADULTANGLERS.rarefied1000.ra), "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

           Df SumsOfSqs MeanSqs F.Model     R2 Pr(>F)
SampleType  6     4.695 0.78248  2.1292 0.1377  0.001 ***
Residuals  80    29.399 0.36749         0.8623
Total      86    34.094                 1.0000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> adonis.SampleType.Station

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ SampleType *      Station, data
= as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                  Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
SampleType         6     4.695 0.78248  3.1799 0.13770  0.001 ***
Station           13    11.619 0.89374  3.6321 0.34078  0.001 ***
SampleType:Station 40    11.137 0.27843  1.1315 0.32665  0.069 .
Residuals         27     6.644 0.24607         0.19487
Total             86    34.094                1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> adonis.SampleType.PelagicZone

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ SampleType *      PelagicZone,
data = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                     Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
SampleType            6     4.695 0.78248 2.13826 0.13770  0.001 ***
PelagicZone           3     2.028 0.67599 1.84726 0.05948  0.002 **
SampleType:PelagicZone 15     4.683 0.31220 0.85315 0.13736  0.950
Residuals             62    22.688 0.36594         0.66546
Total                 86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> adonis.SampleType.AnglerFamily

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ SampleType *      Angler.Family
, data = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                        Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
SampleType               6     4.695 0.78248  2.3342 0.13770  0.001 ***
Angler.Family            3     3.068 1.02281  3.0511 0.09000  0.001 ***
SampleType:Angler.Family 10     3.871 0.38712  1.1548 0.11354  0.073 .
Residuals                67    22.460 0.33522         0.65876
Total                    86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> adonis.SampleType.AnglerTaxa

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ SampleType *      Angler.Taxa,
data = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                       Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
SampleType              6     4.695 0.78248  2.4525 0.13770  0.001 ***
Angler.Taxa             7     5.997 0.85669  2.6851 0.17589  0.001 ***
SampleType:Angler.Taxa 13     4.259 0.32765  1.0270 0.12493  0.407
Residuals              60    19.143 0.31905         0.56148
Total                  86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> adonis.Station

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ Station,      data = as(sample_
data(ADULTANGLERS.rarefied1000.ra), "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

          Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Station   13    12.763 0.98179  3.3599 0.37435  0.001 ***
Residuals 73    21.331 0.29221         0.62565
Total     86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> adonis.Station.SampleType

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ Station *      SampleType, data
= as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                  Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Station           13    12.763 0.98179  3.9899 0.37435  0.001 ***
SampleType         6     3.550 0.59170  2.4046 0.10413  0.001 ***
Station:SampleType 40    11.137 0.27843  1.1315 0.32665  0.064 .
Residuals          27     6.644 0.24607         0.19487
Total              86    34.094                1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> adonis.Station.PelagicZone

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ Station *      PelagicZone, dat
a = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                   Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Station            13    12.763 0.98179  3.4741 0.37435  0.001 ***
PelagicZone         3     0.972 0.32392  1.1462 0.02850  0.214
Station:PelagicZone  3     1.425 0.47500  1.6808 0.04180  0.001 ***
Residuals           67    18.934 0.28260         0.55535
Total               86    34.094                1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> adonis.Station.AnglerFamily

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ Station *      Angler.Family, d
ata = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                     Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Station              13    12.763 0.98179  3.4822 0.37435  0.001 ***
Angler.Family         2     0.781 0.39060  1.3854 0.02291  0.067 .
Station:Angler.Family 1     0.814 0.81382  2.8865 0.02387  0.001 ***
Residuals            70    19.736 0.28194         0.57886
Total                86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> adonis.Station.AnglerTaxa

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ Station *      Angler.Taxa, dat
a = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

            Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Station     13    12.763 0.98179  3.5027 0.37435  0.001 ***
Angler.Taxa  4     1.991 0.49763  1.7753 0.05838  0.001 ***
Residuals   69    19.341 0.28030         0.56726
Total       86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> adonis.PelagicZone

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ PelagicZone,      data = as(sam
ple_data(ADULTANGLERS.rarefied1000.ra), "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

            Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
PelagicZone  3     2.453 0.81782  2.1453 0.07196  0.002 **
Residuals   83    31.641 0.38122         0.92804
Total       86    34.094                1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> adonis.PelagicZone.SampleType

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ PelagicZone *      SampleType,
data = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                      Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
PelagicZone            3     2.453 0.81782 2.23484 0.07196  0.001 ***
SampleType             6     4.269 0.71156 1.94447 0.12522  0.001 ***
PelagicZone:SampleType 15     4.683 0.31220 0.85315 0.13736  0.961
Residuals              62    22.688 0.36594         0.66546
Total                  86    34.094                1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> adonis.PelagicZone.Station

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ PelagicZone *      Station, dat
a = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                   Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
PelagicZone         3     2.453 0.81782  2.8939 0.07196  0.001 ***
Station            13    11.282 0.86782  3.0708 0.33089  0.001 ***
PelagicZone:Station  3     1.425 0.47500  1.6808 0.04180  0.003 **
Residuals           67    18.934 0.28260         0.55535
Total               86    34.094                1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> adonis.PelagicZone.AnglerFamily

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ PelagicZone *      Angler.Famil
y, data = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                         Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
PelagicZone               3     2.453 0.81782  2.3056 0.07196  0.001 ***
Angler.Family             3     2.895 0.96503  2.7206 0.08491  0.001 ***
PelagicZone:Angler.Family 1     0.724 0.72401  2.0412 0.02124  0.014 *
Residuals                79    28.022 0.35471         0.82189
Total                    86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> adonis.PelagicZone.AnglerTaxa

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ PelagicZone *      Angler.Taxa,
data = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                       Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
PelagicZone             3     2.453 0.81782 2.39592 0.07196  0.001 ***
Angler.Taxa             7     5.906 0.84374 2.47184 0.17323  0.001 ***
PelagicZone:Angler.Taxa 1     0.134 0.13413 0.39295 0.00393  0.959
Residuals              75    25.601 0.34134         0.75087
Total                  86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> adonis.AnglerFamily

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ Angler.Family,      data = as(s
ample_data(ADULTANGLERS.rarefied1000.ra), "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

              Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Angler.Family  3     3.155 1.05172  2.8214 0.09254  0.001 ***
Residuals     83    30.939 0.37276         0.90746
Total         86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> adonis.AnglerFamily.SampleType

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ Angler.Family *      SampleType
, data = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                         Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Angler.Family             3     3.155 1.05172  3.1374 0.09254  0.001 ***
SampleType                6     4.608 0.76802  2.2911 0.13516  0.001 ***
Angler.Family:SampleType 10     3.871 0.38712  1.1548 0.11354  0.055 .
Residuals                67    22.460 0.33522         0.65876
Total                    86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> adonis.AnglerFamily.Station

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ Angler.Family *      Station, d
ata = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                     Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Angler.Family         3     3.155 1.05172  3.7303 0.09254  0.001 ***
Station              12    10.389 0.86578  3.0708 0.30472  0.001 ***
Angler.Family:Station  1     0.814 0.81382  2.8865 0.02387  0.001 ***
Residuals             70    19.736 0.28194         0.57886
Total                 86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> adonis.AnglerFamily.PelagicZone

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ Angler.Family *      PelagicZon
e, data = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                          Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Angler.Family              3     3.155 1.05172  2.9651 0.09254  0.001 ***
PelagicZone                3     2.193 0.73113  2.0612 0.06433  0.001 ***
Angler.Family:PelagicZone  1     0.724 0.72401  2.0412 0.02124  0.012 *
Residuals                 79    28.022 0.35471         0.82189
Total                     86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> adonis.AnglerFamily.AnglerTaxa

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ Angler.Family *      Angler.Tax
a, data = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

              Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Angler.Family  3     3.155 1.05172  2.9925 0.09254  0.001 ***
Angler.Taxa    4     3.174 0.79361  2.2581 0.09311  0.001 ***
Residuals     79    27.765 0.35145         0.81435
Total         86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> adonis.AnglerTaxa

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ Angler.Taxa,      data = as(sam
ple_data(ADULTANGLERS.rarefied1000.ra), "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

            Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Angler.Taxa  7     6.330 0.90423  2.5728 0.18565  0.001 ***
Residuals   79    27.765 0.35145         0.81435
Total       86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> adonis.AnglerTaxa.SampleType

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ Angler.Taxa *      SampleType,
data = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                     Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Angler.Taxa           7     6.330 0.90423  2.8341 0.18565  0.001 ***
SampleType            6     4.362 0.72702  2.2787 0.12794  0.001 ***
Angler.Taxa:SampleType 13     4.259 0.32765  1.0270 0.12493  0.421
Residuals            60    19.143 0.31905         0.56148
Total                86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> adonis.AnglerTaxa.Station

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ Angler.Taxa *      Station, dat
a = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

            Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Angler.Taxa  7     6.330 0.90423  3.2260 0.18565  0.001 ***
Station     10     8.424 0.84242  3.0055 0.24709  0.001 ***
Residuals   69    19.341 0.28030         0.56726
Total       86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> adonis.AnglerTaxa.PelagicZone

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ Angler.Taxa *      PelagicZone,
data = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                        Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Angler.Taxa              7     6.330 0.90423 2.64905 0.18565  0.001 ***
PelagicZone              3     2.030 0.67669 1.98244 0.05954  0.003 **
Angler.Taxa:PelagicZone  1     0.134 0.13413 0.39295 0.00393  0.958
Residuals               75    25.601 0.34134         0.75087
Total                   86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> adonis.AnglerTaxa.AnglerFamily

Call:
adonis(formula = DistBC.ADULTANGLERS.rarefied1000 ~ Angler.Taxa *      Angler.Famil
y, data = as(sample_data(ADULTANGLERS.rarefied1000.ra),      "data.frame"))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

            Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Angler.Taxa  7     6.330 0.90423  2.5728 0.18565  0.001 ***
Residuals   79    27.765 0.35145         0.81435
Total       86    34.094                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

APPENDIX 2

Running title: Characterization of the bioluminescent symbionts from ceratioids collected in the Northern Gulf of Mexico

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Keywords: Gulf of Mexico, Ceratioidei, 16S rRNA, bioluminescence, symbiosis

Conflict of Interest: The authors declare no conflicts of interest.

Originality-Significance Statement

This study reports the most comprehensive analysis to date of ceratioid symbionts via molecular methods. Examining the microbial community present within the luminous lure (esca), caruncle, illicium, fin, gill, gut, and skin of adult and larval anglerfishes in addition to seawater collected from the Gulf of Mexico revealed that ceratioid bioluminescent symbionts are not host species specific, are present within seawater, and can be detected at low abundance levels within larval specimens. These findings provide support for the hypothesis that anglerfishes may acquire symbionts from the environment rather than vertically.

Summary

As part of the Gulf of Mexico Research Initiative-funded DEEPEND project (deependconsortium.org), the objective of this study is to characterize the microbiomes of 36 deep-sea anglerfish specimens and identify potential bioluminescent symbiont taxa. Our findings are consistent with previous 16S analysis (Haygood *et al.*, 1992) as well as concurrent results from whole genome sequencing of ceratiids and melanocetids (Hendry *et al.*, 2018). Through the inclusion of additional host species, this study also indicates that Ceratioidei bioluminescent symbionts do not consistently exhibit host specificity at the host family level. In addition to potential bioluminescent symbionts from the family Vibrionaceae, the microbiomes of adult ceratioids contained greater abundance of OTUs representing the non-bioluminescent taxa of the *Moritella* and *Pseudoalteromonas* genera when compared to seawater samples. Adult bioluminescent symbiont OTUs were not found in high abundance within larval ceratioids, however additional Vibrionaceae OTUs

were identified at >10% relative abundance. Future sequencing studies would be beneficial in determining whether these OTUs represent luminous species, as adult conspecifics were largely unavailable for comparison. Lastly, the identification of OTUs representing the bioluminescent symbionts within seawater builds upon recent full genome analysis (Hendry *et al.*, 2018) and provides further support that the ceratioid bioluminescent symbionts may not be obligately dependent upon a host for growth. All of these findings provide support for the hypothesis that ceratioids may acquire their bioluminescent symbionts from the environment.

Introduction

Female anglerfishes belonging to nine of the 11 families within the suborder Ceratioidei develop a lure which is illuminated by bioluminescent bacterial symbionts (Leisman *et al.*, 1980). In the most basic sense, the esca is a spherical, bacteria-filled organ that contains a small opening to the external environment. However, that is not to imply that these organs are simple as they can also contain lenses, filters, and reflectors (Munk, 1999). It is believed that anglerfishes are capable of controlling the bacterial populations within the esca by altering the conditions within the organ (Pietsch, 2009). It is believed that these bioluminescent lures may be used for mate-finding purposes in addition to prey attraction (Herring, 2000, 2007). However, there still remains much speculation regarding the identity of the bioluminescent symbionts and how they are acquired.

Since the symbionts contained within anglerfish escae have historically proven to be unculturable via traditional laboratory techniques, molecular analysis was used by

Haygood and Distel in 1993 to determine the identity of the bioluminescent symbionts. Analysis of the full 16S rRNA gene for two ceratioid species revealed that these symbionts are members of the family Vibrionaceae but are divergent from other known bioluminescent symbionts. In addition, they concluded that the ceratioid symbionts may represent a new bacterial taxa and that the differences between the sequences obtained from each symbiont suggested they may represent two separate bacterial species (Haygood *et al.*, 1992; Haygood and Distel, 1993).

Previous work suggested ceratioid symbionts were unculturable and potentially engaged in an obligate relationship with their hosts (Haygood and Distel, 1993) rather than a facultative relationship as recorded for most marine bioluminescent symbionts (Dunlap and Urbanczyk, 2013). However, typically when an obligate bioluminescent symbiosis has been established, the symbiont is transmitted from the parent generation to the offspring, as the symbiont is dependent upon the host for growth (Dunlap *et al.*, 2007). Such a transmission pathway is not obviously evident based on the life cycle and escal morphology of ceratioids.

Larval anglerfish do not possess a lure capable of housing symbiotic bacteria (Munk and Herring, 1996). It is not until the larvae metamorphose as they make an ontogenetic vertical migration to the depths that the primordial esca invaginates to create a vacuole capable of holding bacteria (Munk *et al.*, 2009; Pietsch, 2009). However it has also been proposed that the female anglerfish may inoculate her eggs with the symbiont before the absorbent and buoyant egg raft makes its way towards the ocean surface where the larvae will hatch (Pietsch, 2009; Fukui *et al.*, 2010; Dunlap *et al.*, 2014). Lastly, the

development of an escal pore suggests that the bioluminescent symbionts are exposed to the external environment (Munk, 1999).

In addition to the esca, females within the family Ceratiidae possess a modified anterior dorsal-fin rays, called a caruncle, which is similar in form to the esca. Members of the genus *Ceratias* develop two caruncles, while members of the genus *Cryptopsaras* develop three caruncles. Histological study of a *C. couesii* caruncle has concluded that like the esca, dense populations of luminous bacteria are present and can be expelled through a distal pore (Hansen and Herring, 1977; Herring and Morin, 1978).

With this study we aim to characterize the microbial communities found within the bioluminescent organs of both adult and larval anglerfishes in order to discern greater detail regarding the symbiotic relationship between anglerfishes and their bioluminescent bacteria. Seawater samples from the Gulf of Mexico will also be examined for the presence of potential symbiont taxa to explore the likelihood of escal bioluminescent symbionts being acquired from the environment.

Results

A total of 36 anglerfish specimens were collected over the course of four DEEPEND cruises aboard the *R/V Point Sur* in the Gulf of Mexico: DP01 from May 1 – 8, 2015, DP02 from August 8-21, 2015, DP03 from April 20 – May 14, 2016, and DP04 from August 5-19, 2016. These specimens consist of adult and larval individuals belonging to six of the families with the suborder Ceratioidei: Ceratiidae (n=22), Oneirodidae (n=7), Linophrynidae (n=3), Melanocetidae (n=2), Centrophrynidae (n=1), Gigantactinidae (n=1).

Community Analysis

Anglerfish specimens were examined by organ type in comparison to each other and to the water samples. Significant differences were found in the microbial community richness and diversity between anglerfish and water specimens (Figure 1'). The observed richness (ANOVA, df=7, F=68.15, p=<0.001) and Chao1 index (ANOVA, df=7, F=40.76, p=<0.001) showed significant differences in richness and diversity among sample types. Diversity as measured by the Shannon index (ANOVA, df=7, F=89.5, p=<0.001) and Inv. Simpson index (ANOVA, df=7, F=20.51, p=<0.001) also showed significant differences among sample types. These significant results were driven primarily by differences between the anglerfish and water samples (Supplemental Table 4). NMDS visualization of the data revealed a distinct clustering of water samples while all anglerfish organ types overlapped (Figure 2'). Permuted multivariate ANOVA (Adonis) analysis showed that examining anglerfish specimens at the organ level to water provided a slightly greater explanation as this accounts for 17% of the variation (PERMANOVA, df=7, F=9.09, R^2=0.17, p=0.001). SIMPER analysis revealed that OTUs 112983 (*Moritella* sp.), 830290 (*Pseudoalteromonas* sp.), 9131 (*Enterovibrio* sp.), and 792393 (*Vibrio shilonii*) were driving the significant differences between anglerfish and water microbiomes accounting for 15.5%, 9.5%, 8.8%, and 6.7% of the differences respectively.

Although most abundant overall, Vibrionaceae were primarily found within the caruncle and escal specimens, but were not limited solely to the bioluminescent organs (Figure 3'). Members of the family Moritellaceae are present in highest abundance on the

fins, skin, and guts while Pseudoalteromonadaceae is most abundant within escal and illicial organs (Figure 3').

Water samples were then excluded in order to directly compare the microbial richness and diversity of anglerfish organ types to one another. Significant differences in the microbial community richness and diversity were found between anglerfish organ types as measured by the Shannon index (ANOVA, df=6, F=2.204 p=0.048) and Inv. Simpson index (ANOVA, df=6, F=2.244, p=0.044). These significant results were driven by differences between the guts and esca (InvSimpson, Tukey's HSD P=0.022) as well as guts and skin (Inv. Simpson, Tukey's HSD P=0.025).

Potential Symbiont Taxa in Adult Escal and Caruncle Specimens

Sequencing revealed five potential bioluminescent symbiont taxa (OTU IDs: 9131, 160210, 9129, 523223, 939811). All taxa belong to the family Vibrionaceae and accounted for greater than 10% of the relative abundance. OTUs 9129, 160210, and 939811 could only be identified to the family level as Vibrionaceae while OTU 9131 was placed within the genus *Enterovibrio*. OTU 523223 clustered at >97% identity to *Photobacterium angustum*. While most strains of *Photobacterium angustum* are not known to exhibit bioluminescence, OTU 523223 will be considered a potential bioluminescent symbiont as the luminous strain GB-1 has been provisionally included within the species (Urbanczyk *et al.*, 2010).

OTU ID 9131 was identified with a relative abudance greater than 10% in nine escal specimens (all belonging to *C. couesii* hosts) (Figure 4'). While OTUs 9129 and 160210 were abundant within the escal specimens belonging to hosts within Melanocetidae and Oneirodidae families. Within the escal specimens from both

undefined *Ceratias* individuals, OTU 939811 was the most abundant potential bioluminescent symbiont. No bioluminescent potential symbiont OTU was found at a relative abundance greater than 10% in seven of the 21 escal specimens and three of the nine caruncle specimens. However, more in depth analysis revealed that at least one of the five potential bioluminescent symbiont taxa were present within each specimen. No adult escal or caruncle specimens were entirely devoid of a potential symbiont taxa (Supplemental Table 7, Supplemental Table 8).

OTU ID 9131 was identified within four of nine caruncle specimens with a relative abundance ranging from 45.6% - 98.8% (all *C. couesii* hosts). OTU IDs 9121 and 160210 were found within the caruncle specimens of an unknown host belonging to the genus *Ceratias*. Lastly, OTU 523223, which was not present in high abundance within the escal specimen of the same host nor within the escal specimens of other host species, was identified with in the caruncle of a *C. couesii* host.

Of the seven *C. couesii* specimens from which an escal and caruncle sample were processed, five showed similar patterns of OTU abundance within both organ types. As stated above, individual CC57 contained OTU 523223 in an abundance greater than 10% within the caruncle but not within the esca. Specimens CC71.N0 and CC79.2 did not contain a high abundance of a potential bioluminescent symbiont OTU in either organ type.

Potential Symbiont Taxa in Larval Escal and Caruncle Specimens

Larval anglerfish specimens were also collected, and sequencing revealed six potential bioluminescent symbiont taxa (OTU IDs: 523223, 939811, 136178, 176420, 792393, 837366). All taxa belong to the family Vibrionaceae and account for greater than

10% of the relative abundance within any organ type of a larval specimen. OTUs 523223 and 939811 were also identified within specimens from adult anglerfishes, but the other OTUs identified within larval specimens were not seen in high abundance within the adults. OTUs 136178, 176420 and 939811 could only be identified to the family level as Vibrionaceae while OTU 523223 and OTU 792393 clustered at >97% identity to *Photobacterium angustum* and *Vibrio shilonii,* respectively.

OTU ID 523223 was identified with a relative abudance greater than 10% in just one larval specimen which did not have a visible esca. OTU 136178 was present within the escal specimens of a larval Linophrynidae and a larval Oneirodidae specimen. OTU 176420 was present in high abundance within only one specimen, an esca from a Linophrynidae larva. 939811 was also present in only one specimen, an esca from an Oneirodidae larva. Lastly, OTU 792393 was the most abundant across all larval escal specimens with a relative abundance ranging from 11.1% to 66.8% across six of the 13 samples, but is unlikely to be a bioluminescent symbiont as it is not luminescent based on taxonomic assignment.

While none of the three most likely OTUs identified as potential bioluminescent symbionts (9131, 9129, and 160210) within the adult anglerfish specimens were present with a relative abundance level greater than 10% in the larval specimens, at least one of the three taxa was present at a very low level in all but two larval escal or caruncle specimens (Supplemental Table 1', Supplemental Table 2').

Presence of Potential Symbiont Taxa in Seawater Specimens

All eight potential symbiont OTUs were detected in at least 41 of the 214 seawater samples at low relative abundance levels ranging from 0 - 0.66% per sample.

OTU 523223 was most abundant across all seawater samplesd followed by OTUs 939811, 9131, 176420, 837366, 136178, 160210, and 9121 respectively. However, when examined by depth, symbiont OTUs were on average most abundant within the mesopelagic and bathypelagic zones (Figure 5').

Discussion

Anglerfish and Seawater Microbiomes

Not unlike the findings of prior studies on fish-associated microbiomes and their environment (Larsen *et al.*, 2015; Legrand *et al.*, 2018; Pratte *et al.*, 2018), there exists a significant difference in the richness and diversity of the microbial community found within all tested organ types of the anglerfish specimens and the surrounding environment (Figure 1'). The greatest difference between the two is the greater abundance of the genera *Moritella*, *Pseudoalteromonas*, *Enterovibrio*, and *Vibrio* within anglerfish specimens as compared to the water.

OTU 112983 represents an unknown species within the genus *Moritella* and was present at high abundance levels within all organs of adult anglerfishes. Members of the genus *Moritella* are generally piezophilic are suspected to form mutualistic relationships with deep-sea organisms (Urakawa, 2013). One member of the genus, *M. viscosa*, is known to cause skin ulcerations in fish (Urakawa, 2013). Also present at high abundance levels within the escae and illicia of adult anglerfishes was OTU 830290 representing the genus *Pseudoalteromonas.* Known members of *Pseudoalteromonas* have been reported to provide antifouling and/or algicidal benefits (Holmström and Kjelleberg, 1999). More detailed investigation may be beneficial to determine if the taxa identified here also

exhibit antifouling properties which may in turn aid the host in reducing the presence of microbes that compete with or prevent colonization by bioluminescent symbionts. Lastly, the genera *Enterovibrio* and *Vibrio* contain bioluminescent species known to form symbiotic relationships with host organisms (Dunlap and Urbanczyk, 2013).

Microbial Communities – Adult Anglerfishes

Examining adult anglerfish specimens by organ type did not reveal any significant differences in regards to microbial richness or diversity. However, the escae and caruncles of adult anglerfishes had the lowest levels of microbial richness and diversity in comparison to other organ types sampled. The lack of significant difference may be in part due to the fact that the entire bioluminescent organ was processed, including the outer epithelial surface. Including the outer skin of the organ in the extraction process may have inflated the diversity and richness of these organs.

Bray-Curtis dissimilarity analysis revealed that the collection site (station) accounted greatest percentage of variation seen within adult anglerfish specimens. This was primarily driven by the high abundance of *Moritella sp.* present in samples collected from stations SW5 and B175. However, samples were unevenly sampled across stations, so it is difficult to draw any strong conclusions. Host species accounts for second greatest percentage of variation seen within adult anglerfish microbial communities. Several previous studies have indicated that host species plays a significant role in the microbial community of fish(Larsen *et al.*, 2013; Boutin *et al.*, 2014; Pratte *et al.*, 2018). These findings indicate that the microbiome of adult anglers is influenced in part by the environment, but may also regulated by host specific relationships with microbes.

Microbial Communities– Larval Anglerfishes

Like adults, collection location (station) explained the greatest percentage of variation within the microbial communities of larval anglerfishes. However, collection depth was the second strongest driver of beta diversity. Unfortunately due to the nature of sample collection, a large portion of larval specimens were collected from net N0 which collects samples throughout the entire descent from the surface to the maximum depth of 1500m so we are unable to discern at which discrete depth the specimen was collected. These samples were binned together and thus reduces the strength of this observation.

Adult Anglerfish Bioluminescent Symbionts

The bioluminescent organs of adult anglerfishes were dominated by OTUs 9131, 160210, and 9129, with OTUs 523223 and 939811 also present, but less distinct. Our results indicate a potential host-species specific symbiotic relationship between *C. couesii* host and symbiont OTU 9131. This is supported by previous 16S sequencing as well as current full genome sequencing of the *C. couesii* bioluminescent symbiont (Haygood *et al.*, 1992; Hendry *et al.*, 2018).

However, symbiont analysis also indicated the possibility of dual symbionts within the bioluminescent organs of two Melanocetidae, one *Dolopichthys*, and an unknown *Ceratias* host. Where present, OTUs 160210 and 9129 appear together in high abundance. Previous study of the *M. johnsonii* symbiont matches to OTU 9129 and current full genome sequencing of the *M. johnsonii* bioluminescent symbiont indicates a single symbiont species(Hendry *et al.*, 2018). Therefore, OTU 160210 may be a remnant of the OTU picking process and not necessarily a secondary symbiont taxon.

OTU 523223 was found in high abundance within the caruncle of a single *C. couesii* specimen while OTU 939811 was identified within the escae of both undefined *Ceratias* specimens. However, these potential symbiont OTUs are present at fairly high abundance levels within other organ types. It is unclear from this analysis whether these OTUs are indeed bioluminescent symbionts cultured for the purpose of illuminating the anglerfishes' escae. Future full genome sequencing may help to shed light on the likelihood that these taxa represent a bioluminescent symbiont.

For the *C. couesii* specimens from which a caruncle and escal specimen were collected, one of the identified potential symbiont OTUs appeared in high abundance within both organ types. This confirms prior observations of bioluminescent bacteria possibly oozing from the caruncles of freshy collected specimens (Pietsch, 2009) and indicates that the same symbiont taxa is cultivated by the host in both luminous organs. It has also been hypothesized that the illicium may provide a way for the bioluminescent symbiont to be transferred from the caruncle to the esca (Pietsch, 2009), but OTU 9131 was not identified at high abundance levels within the illicia of adult *C. couesii* individuals. Since the *C. couesii* symbiont (OTU 9131) was not detected at >10% relative abundance within the illicia for any *C. couesii* specimen for which an escal and caruncle specimen was also processed, it is concluded that the illicium does not provide a continuous means for symbiont transport between the caruncle and esca of adult *C. couesii*.

Larval Anglerfish Bioluminescent Symbionts

Without an adult specimen of the same species with which to compare, we cannot draw many strong conclusions regarding bioluminescent symbionts within larvae, but it is

294 worth noting that OTU 9131, which was found in high abundance within adult *C. couesii*
295 anglerfishes, was identified at lower relative abundance levels (0.01-0.11%) within the
296 primordial escae and caruncles of the three larval *C. couesii* specimens. The presence of
297 the symbiont OTU supports the hypothesis that the larvae may have been inoculated by
298 their mother (Pietsch, 2009). However, the relative abundance level of OTU 9131within
299 *C. couesii* larval specimens was not dramatically greater than the relative abundance of
300 OTU 9131 within seawater samples (0 – 0.66%). Without a more controlled comparison,
301 it is difficult to definitively conclude that the symbiont detected within the larval samples
302 is due to either vertical transmission or environmental acquisition. It should also be noted
303 that these larvae were collected at depths between 10m and 999m so it is possible that the
304 larvae had already begun their ontogenetic vertical migration.
305 The potential symbiont OTUs identified at high abundance in the escal specimens
306 of larvae (523223, 939811, 136178, 176420, 837366) were also found at abundance
307 levels >10% in at least one other organ type. This may be an indication that the
308 bioluminescent symbiont is not limited solely to the escal region in larval anglerfishes, or
309 that non-bioluminescent members of the family Vibrionaceae are also present at high
310 abundance levels in larvae. Full genome sequencing of potential larval symbionts as well
311 additional sampling and analysis of corresponding adults would aid in clarifying this
312 observation.

313 *Bioluminescent Symbionts within Seawater*

314 To examine the possibility that the larvae may be acquiring symbionts from their
315 environment, we searched for the potential symbionts within seawater samples. Traces of
316 all eight potential symbionts were found within the water at very low levels of relative

abundance. This finding may imply that the bioluminescent symbionts of ceratioids are not obligately dependent for growth as they are able to survive outside of the host and therefore are more likely to be acquired from the environment as is seen in other symbiotic relationships between bioluminescent bacteria and fishes (Dunlap and Urbanczyk, 2013). These findings are also supported by the recent full genome analysis of the *C. couesii* bioluminescent symbiont, which indicated that the symbiont has retained motility genes required for development of a flagellum (Hendry *et al.*, 2018). In addition, all eight potential symbionts were found at the greatest abundance within the mesopelagic and bathypelagic zones. A greater concentration of these OTUs at depth also supports the hypothesis that larval anglerfishes acquire bioluminescent symbionts from the environment as the esca develops and the larvae make their ontogenetic migration from the surface waters to the bathypelagic zone (Pietsch, 2009).

Experimental Procedures

Sample Collection and Processing

All anglerfish and seawater samples were collected over the course of four cruises aboard the *R/V Point Sur* in the Gulf of Mexico: DP01 from May 1 – 8, 2015, DP02 from August 8-21, 2015, DP03 from April 20 – May 14, 2016, and DP04 from August 5-19, 2016. Previously established SEAMAP station locations were used for labeling collection sites (www.gsmfc.org). All anglerfish specimens were collected using a 10 m^2 mouth area, six-net MOCNESS (Multiple Opening and Closing Environmental Sensing System) with 3-mm mesh (Wiebe *et al.*, 1976).

Water samples were also collected at each station using a separate CTD cast. During each cast, Niskin bottles were fired at a maximum of five targeted depths based on depth, chlorophyll *a* fluorescence, or dissolved oxygen levels. Four to five liters of seawater were collected from each sampled depth and separated into three one-liter replicates that were then filtered through a 0.45-micron filter (Daigger) under low pressure using a vacuum pump (Easson and Lopez, 2018, in review). All specimens were stored at -80C until processed by the Microbiology & Genetics Laboratory at Nova Southeastern University's Halmos College of Natural Sciences and Oceanography. Reports for each of the four cruises can be found at the following sites: http://www.deependconsortium.org/images/documents/DP01_report.pdf, http://www.deependconsortium.org/images/documents/DP02_CruiseReport.pdf, http://www.deependconsortium.org/images/documents/DP03_CruiseReport.pdf, and http://www.deependconsortium.org/images/documents/DP04_Cruise_Report.pdf.

Specimen Taxonomy

Once onboard, anglerfish specimens were sorted, identified to the lowest taxonomic level possible, and placed in ethanol or RNALater by DEEPEND Consortium's Chief Scientist Dr. Tracey Sutton (Sutton *et al.*, 2010; Pietsch and Sutton, 2015).

Microbial DNA Extraction

Anglerfish specimens were dissected with sterilized instruments. For specimens collected during cruises DP01 and DP02, the entiring luring apparatus (esca and illicium) were dissected as a single sample labeled as esca. Lure samples collected during the later cruises (DP03 and DP04), were split into two separate specimens labeled as the esca and

362 illicium accordingly. For Ceratiid specimens, the base of the caruncles was separated
363 from the back of the fish and all two or three caruncles, depending on anglerfish species,
364 were included in the sample. The least damaged pectoral fin was dissected as well as an
365 undamaged portion of skin from the lateral side of the anglerfishes. For gill sample
366 dissection, the gill-filaments, gill-rakers, and gill arch were removed from one side of the
367 anglerfish. Lastly, the entire intestine, from the base of the stomach to the cloaca was
368 extracted for the gut sample.

369 All microbial DNA isolations were conducted following the Earth Microbiome
370 Project (earthmicrobiome.org) protocol with the MO BIO PowerLyzer™ PowerSoil® kit.
371 After extraction a 1% agarose gel was run to ensure that the DNA extraction was
372 successful. After gel verification the DNA concentration was confirmed using the Qubit
373 2.0 (Life Technologies).

374 *Illumina High-Throughput Metagenomic Sequencing*

375 All samples were prepared for sequencing following the 16S Illumina Amplicon
376 Protocol per the Earth Microbiome Project (Caporaso *et al.*, 2011). The 806R and 515F
377 primers were used for PCR amplification of the V4 region of the 16S rRNA gene
378 (Caporaso *et al.*, 2011). Amplicons were sequenced with an Illumina MiSeq using the V2
379 500-cycle cartridge across three runs to generate paired-end 250 base pair amplicons
380 (Caporaso *et al.*, 2012).

381 *Sequencing Analysis: QIIME*

382 The initial processing of raw microbiome data was performed using Quantitative
383 Insights into Microbial Ecology (QIIME) version 1.9.1 (Caporaso *et al.*, 2010). The
384 forward and reverse paired-end reads were joined and converted to FASTA files using

385 "join_paired_ends.py" with the default settings. Sequences were then demultiplexed and
386 quality filtered (quality score > 29) using "split_libraries_fastq.py." Lastly, sequences
387 were clustered into operational taxonomic units (OTUs) based on 97% similarity using
388 the default settings for "pick_open_reference_otus.py." Taxonomic classification was
389 assigned via the GreenGenes database (DeSantis *et al.*, 2006; Caporaso *et al.*, 2010).

390 *Community Analysis: R*

391 Analysis was executed with the RStudio software (version 3.2.1, (R Core Team,
392 2016), with the added packages 'phyloseq' and 'vegan' to examine general microbial
393 ecology (McMurdie and Holmes, 2013; Oksanen *et al.*, 2018). Seawater replicates were
394 merged into a single sample per collection depth and location. All samples were then
395 rarefied to a uniform depth of 1000 sequences and were transformed to reflect relative
396 abundance. Variations associated with sample type (anglerfish or water), organ type
397 (esca, caruncle, illicium, fin, gill, gut, or skin), and anglerfish developmental stage
398 (larval, post-larval, or adult were analyzed using these tools.

399 Alpha diversity was measured by calculating OTU observed richness, Chao1
400 index, Shannon index, and the Inverse Simpson's index for each sample type, anglerfish
401 organ type, and anglerfish developmental stage using phyloseq (McMurdie and Holmes,
402 2013). Differences in alpha diversity among sample type, organ type, and developmental
403 stage were assessed using an analysis of variance (ANOVA) followed by the post hoc
404 test, Tukey's Honest Significant Difference (HSD) to determine pairwise differences.

405 Beta diversity was measured by calculating Bray-Curtis dissimilarity to determine
406 differences in the community composition by sample type, anglerfish organ type, and
407 anglerfish developmental stage. Dissimilarity was presented as distance matrices and a

permuted multivariate ANOVA (Adonis) was used to assess significant differences. Lastly, a SIMPER test with 499 permutations was used to show which specific taxa were driving differences between sample type and organ type microbiomes.

Symbiont Analysis: R

For symbiont analysis, the original, unrarefied dataset was used so as not to exclude rare taxa that may have been inadvertently excluded when normalizing to a uniform depth of 1000 sequences. For this dataset, 16S rRNA sequence data was transformed to reflect relative abundance. The most abundant OTUs (relative abundance >10%) were examined within escal and caruncle samples of adult anglerfish samples to identify potential bioluminescent symbiont taxa. These were then filtered for members belonging to the family *Vibrionaceae*, which contains known bioluminescent symbionts of fishes (Dunlap and Urbanczyk, 2013). A phylogenetic tree for the most abundant OTUs (relative abundance >10%) was also generated to verify that any taxa not classified to the family level were not excluded unintentionally. Once potential bioluminescent symbiont taxa were identified within adult anglerfish samples, larval anglerfish samples of matching species were examined for identical OTUs. The same process to identify potential symbionts in the adult anglerfish samples was used to identify additional potential symbionts within larval specimens for which an adult specimen of the same species was not available. Lastly, the relative abundance of these potential symbiont taxa was determined within other anglerfish organ types and within water samples.

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Table and Figure Legends

Figure 1'. Boxplot of species richness and diversity comparing sample types based on observed richness (ANOVA, df=7, F=68.15, p=<0.001), Chao1 index (ANOVA, df=7, F=40.76, p=<0.001), Shannon index (ANOVA, df=7, F=89.5, p=<0.001), and Inverse Simpson index (ANOVA, df=7, F=20.51, p=<0.001).

Figure 2'. Non-metric dimensional scaling of anglerfish and water samples. (R^2 = 0.97, stress= 0.1699, solid ellipse = multivariate normal distribution with 95% CI).

Figure 3'. Bar plot of taxa present at greater than 10% relative abundance within adult anglerfish specimens by Family.

Figure 4'. Bar plot of taxa present at greater than 10% relative abundance within adult anglerfish specimens by OTU ID.

Figure 5'. Heatmap of relative abundance of all potential symbiont OTUs in seawater by Depth Zone

Supplemental Table 1'. Anglerfishes collected for microbiome analysis. Abbreviations for sampled organs: caruncle (c), esca (e), fins (f), illicium (i), gills (g), guts (gu), and/or skin (s).

Supplemental Table 2'. Water samples collected for microbiome analysis.

Tables and Figures

Figure 1'.

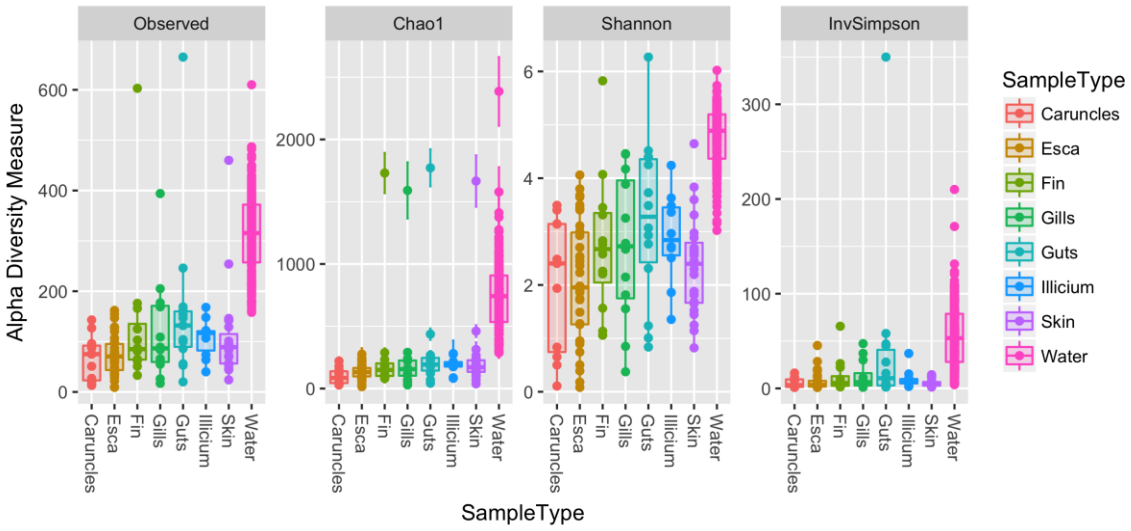


Figure 2'.

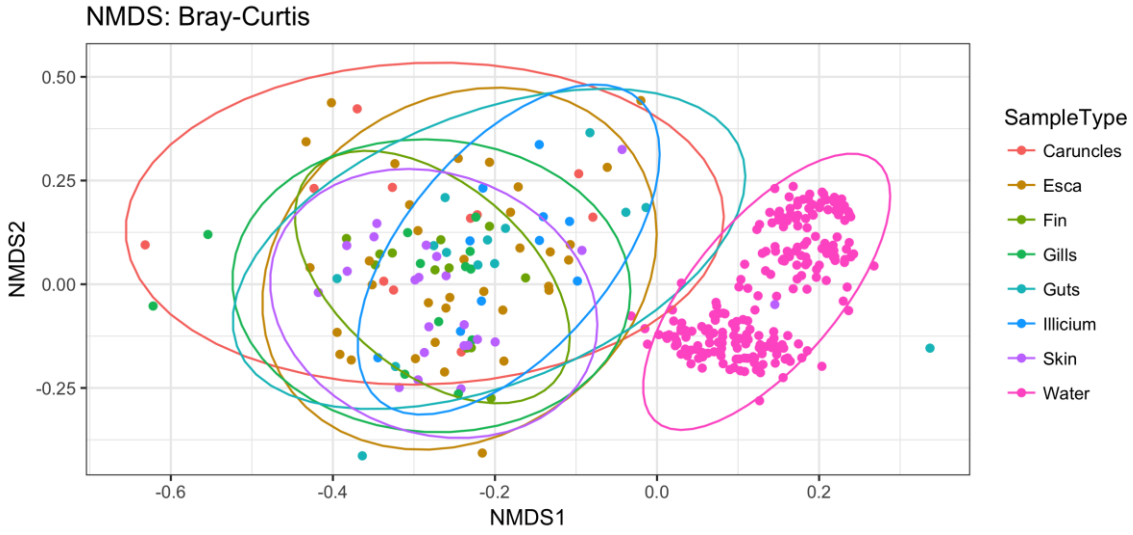


Figure 3'.

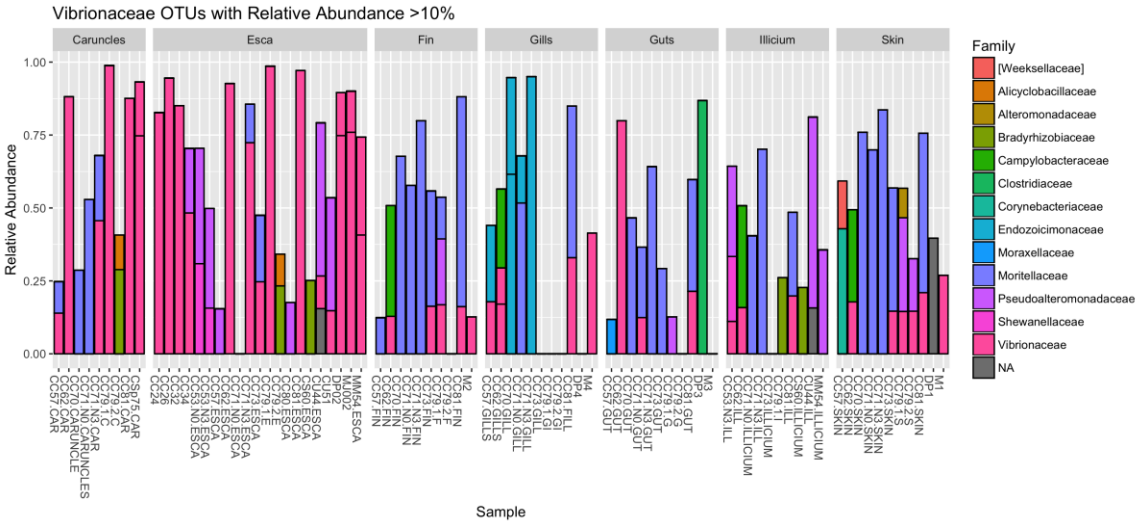


Figure 4'.

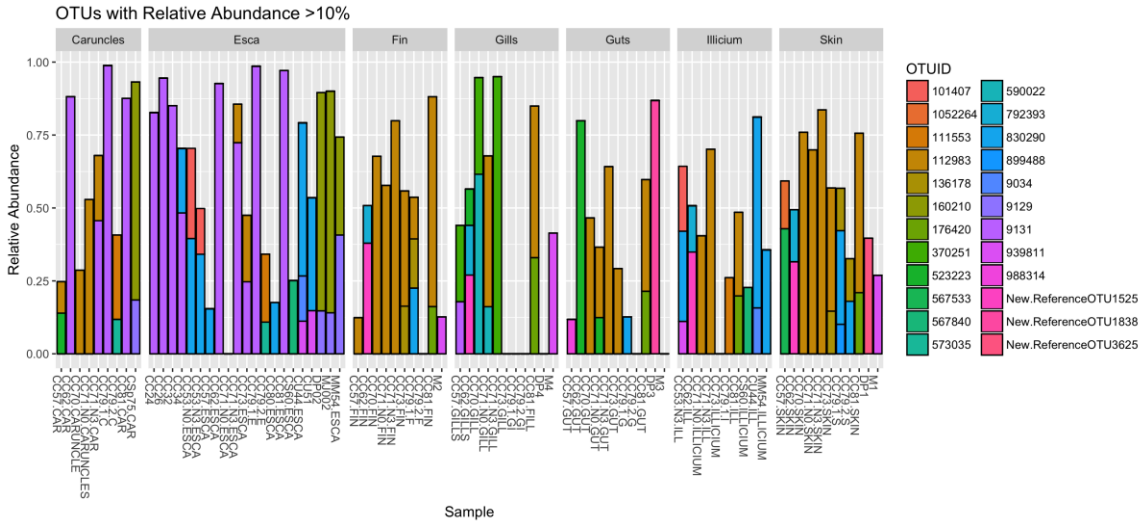
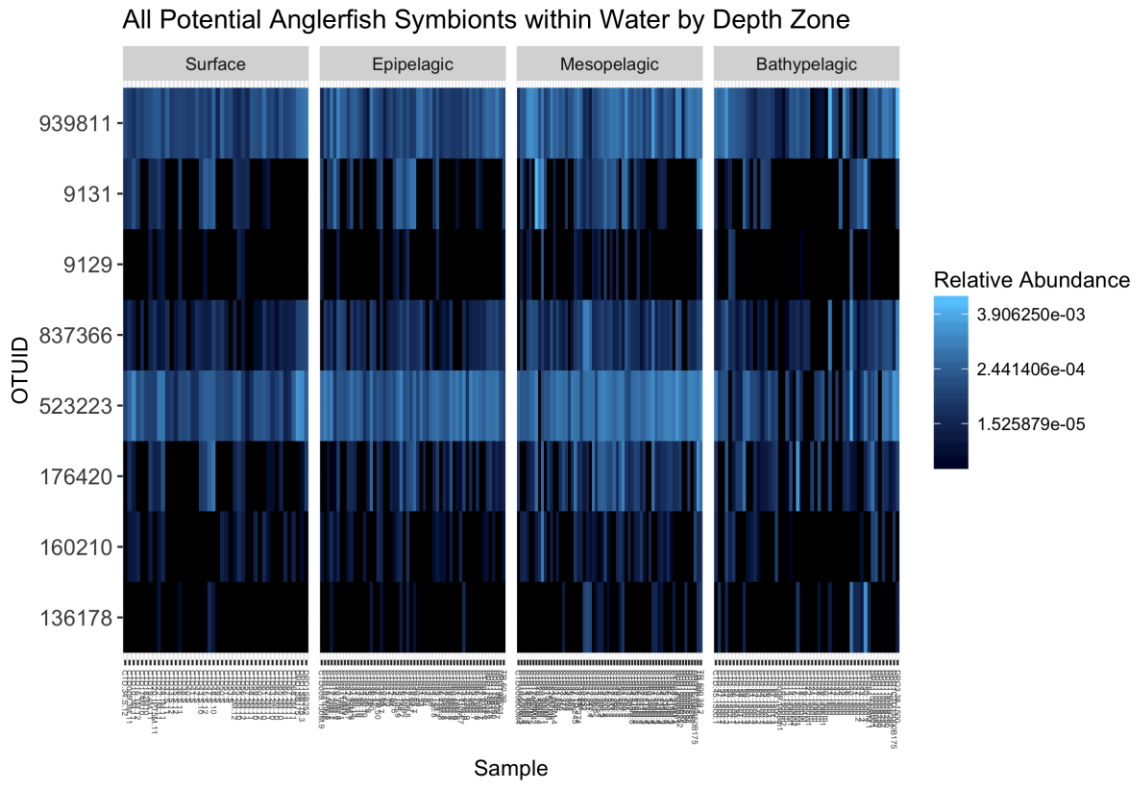


Figure 5'.



492 Supplemental Table 1'.

ID	Taxonomy (Family, species)	Dev. Stage	Organs sampled	Cruise	Station	Trawl #	Trawl Depth (m)
DP02	Oneirodidae *Dolophichys* sp.	Adult	e, g, gu, s	DP01	B001	02	0-1201
MJ02	Melanocetidae *Melanocetus johnsonii*	Adult	e, f, g, gu, s	DP01	B001	03	0-1143
CC24	Ceratiidae *Cryptopsaras couesii*	Adult	e	DP02	B252	24	600-198
CC26	Ceratiidae *Cryptopsaras couesii*	Adult	e	DP02	B080	26	0-751
CC32	Ceratiidae *Cryptopsaras couesii*	Adult	e	DP02	SE3	32	597-198
CC34	Ceratiidae *Cryptopsaras couesii*	Adult	e	DP02	B255	34	1000-600
CC42	Ceratiidae *Cryptopsaras couesii*	Larva	c, e, s	DP03	B003	42	998-599
CC53.N0	Ceratiidae *Cryptopsaras couesii*	Adult	e	DP03	B081	53	11-1504
CC53.N3	Ceratiidae *Cryptopsaras couesii*	Adult	e, i	DP03	B081	53	1002-601
CU44	Undefined *Ceratias* sp.	Adult	e, i	DP03	B079	44	997-601
CU51	Undefined *Ceratias* sp.	Adult	e	DP03	B252	51	11-1502
MM54	Melanocetidae *Melanocetus murrayi*	Adult	e, i	DP03	B081	54	11-1500
CC57	Ceratiidae *Cryptopsaras couesii*	Adult	c, e, f, g, gi, s	DP04	SW6	57	10-924
LI58	Unknown Linophrynidae sp.	Larva	e, s	DP04	SW6	58	1515-1203
CC59	Ceratiidae *Cryptopsaras*	Larva	e	DP04	SW6	59	202-10

	couesii						
GI59	Unknown Gigantactinidae sp.	Larva	e, s	DP04	SW6	59	10-1500
LI59	Unknown Linophrynidae sp.	Larva	e, s	DP04	SW6	59	1498-1201
CC60	Ceratiidae *Cryptopsaras couesii*	Larva	c, e, f, g, gu, s	DP04	SW4	60	999-602
CS60	Centrophrynidae *Centrophryne spinulosa*	Adult	e, i	DP04	SW4	60	999-602
ON62.1	Unknown Oneirodidae sp.	Larva	e, s	DP04	SE1	62	11-1499
CC62	Ceratiidae *Cryptopsaras couesii*	Adult	c, e, i, f, g, gu, s	DP04	SE1	62	11-1499
ON62.2	Unknown Oneirodidae sp.	Larva	e, s	DP04	SE1	62	11-1499
ON64	Unknown Oneirodidae sp.	Larva	e, s	DP04	SE3	64	11-1501
ON69	Unknown Oneirodidae sp.	Larva	e, gu, s	DP04	SW3	69	998-601
CC70	Ceratiidae *Cryptopsaras couesii*	Adult	c, f, g, gu, s	DP04	SW5	70	998-600
CC71.N0	Ceratiidae *Cryptopsaras couesii*	Adult	c, e, f, g, gu, i, s	DP04	SW5	71	11-1505
CC71.N3	Ceratiidae *Cryptopsaras couesii*	Adult	c, e, f, g, gu, i, s	DP04	SW5	71	1001-593
CC73	Ceratiidae *Cryptopsaras couesii*	Adult	e, f, g, gu, i, s	DP04	B064	73	11-1512
ON76	Unknown Oneirodidae sp.	Post Larva	e, f, g, gu, s	DP04	B065	76	1000-599
LI78	Unknown Linophrynidae sp.	Larva	e, s	DP04	B287	78	996-603
ON78	Unknown Oneirodidae sp.	Larva	e, s	DP04	B287	78	11-1501
CC79.1	Ceratiidae *Cryptopsaras couesii*	Adult	c, e, f, g, gu, i, s	DP04	B252	79	1001-605

CC79.2	Ceratiidae *Cryptopsaras couesii*	Adult	c, e, f, g, gu, s	DP04	B252	79	1001-605
CC80	Ceratiidae *Cryptopsaras couesii*	Adult	e	DP04	B252	80	10-1500
CC81	Ceratiidae *Cryptopsaras couesii*	Adult	c, e, f, g, gu, s	DP04	B175	81	1000-600

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494 Supplemental Table 2'.

Cruise	**CTD Cast #**	**Station**	**Depth(m)**
DP01	1	B001	1000, 450, 50, 2
DP01	2	B175	1000, 450, 2
DP01	3	B175	75, 35
DP01	4	B252	400, 30
DP01	5	B287	1600, 475
DP01	6	B287	95, 75
DP01	7	B082	1600, 465, 65
DP01	8	B250	1600, 1000, 450, 75
DP02	9	SW4	1466, 600, 130, 1
DP02	10	SW4	1500, 650, 110, 1
DP02	13	SE1	1500, 750
DP02	14	B286	1490, 660
DP02	16	B287	1507, 467, 90, 1
DP02	17	B252	1500, 462, 70, 1
DP02	18	B175	1500, 1404, 40, 1
DP02	19	B175	1404, 399, 1
DP02	20	B080	800, 498, 73, 1
DP02	21	B080	800, 500, 43, 12
DP02	22	B003	1510, 457, 72, 1
DP02	24	B079	1510, 600, 92, 1
DP02	27	SE4	1499
DP02	28	SE4	1500
DP02	29	B255	1496
DP02	30	B255	1500
DP03	31	B082	1600, 456, 80

DP03	32	B082	1600, 450, 80, 2
DP03	33	B082	1500, 377, 68, 2
DP03	34	B082	1600, 375, 50, 2
DP03	35	B287	1500, 303, 56, 2
DP03	36	B287	1500, 283, 160, 52, 2
DP03	37	B287	274, 245, 50
DP03	38	B003	1500, 244, 59, 2
DP03	39	B003	300, 50
DP03	40	B003	1500, 252, 64, 2
DP03	41	B079	1500, 237, 70, 2
DP03	42	B079	1500, 347, 94, 2
DP03	43	B079	1500, 360, 86, 2
DP03	44	B079	300, 50
DP03	45	SE4	1500, 533, 145, 105, 2
DP03	46	SE4	300, 50
DP03	47	SE5	1500, 511, 106, 2
DP03	48	B252	396, 64, 2
DP03	49	B252	360, 49, 2
DP03	50	B081	1500, 467, 49, 2
DP03	51	B081	1500, 480, 53, 2
DP03	52	B175	1500, 485, 54, 2
DP03	53	B175	507, 59, 2
DP04	54	SW6	1499, 545, 130, 2
DP04	55	SW6	1502, 516, 125, 2
DP04	56	SW4	1500, 446, 43, 2
DP04	57	SE1	1495, 441, 68, 2
DP04	58	SE3	1501, 444, 90, 2
DP04	59	SE3	1500, 418, 86, 2
DP04	60	SE2	1500, 386, 86, 2
DP04	61	SW3	1500, 359, 76, 2
DP04	62	SW5	1500, 498, 110, 2
DP04	63	B064	1520, 421, 97, 2
DP04	64	B064	1500, 415, 95, 22, 2
DP04	65	B065	1500, 334, 58, 2
DP04	66	B287	1503, 340, 70, 2
DP04	67	B252	1501, 415, 80, 2
DP04	68	B175	1500, 374, 51, 2

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