Adolescence and the desire to fit in: behavior and mimicry in the juvenile and subadult life stages of the mesopelagic squid, *Chiroteuthis calyx*

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ABSTRACT

Despite the fact that cephalopods are very common in the mesopelagic zone of our deep oceans, very little is known about their behavior due to the inaccessibility of this environment. Recent studies suggest that, contrary to historical predictions, deep-sea cephalopods exhibit a wide array of visually linked behaviors. In this study, we used *in situ* footage from Remotely Operated Vehicles, coupled with laboratory observations to construct a behavioral ethogram for the mesopelagic squid, *Chiroteuthis calyx*. 50 juvenile and 28 subadult *C. calyx* were observed in order to construct an this ethogram that describes and classifies 73 behavioral components and 5 behavioral sequences. While the exact function of these behaviors remains unknown, we offer speculations in correspondence with the selective forces of their habitat. Juvenile *C. calyx* have long been noted to be morphologically similar to the abundant and active siphonophore, *Nanomia bijuga*, that occurs in the same habitat and depth range as *C. calyx*. A behavioral comparison was made between the juvenile and subadult life stages by randomly resampling the frequency of behaviors in both age cohorts. We also offer

evidence for the juvenile's hypothesized mimicry of *N. bijuga* by comparing postural components of the behaviors of both species in an orientation study.

INTRODUCTION

The pelagic habitat of the deep sea is the largest and least explored environment on our planet (Robison, 2004). The dimly-lit subsection of this habitat called the mesopelagic, midwater, middle pelagic, or twilight zone comprises a vast swath of our oceans, occupying the depths from 200 to 1000 m worldwide (Robison, 1999; Robison, 2004; Bush, et al., 2009). The selective pressures that characterize the mesopelagic habitat include cold temperatures, low food levels, high pressures, low oxygen content, minimal to nonexistent sunlight illumination, and increased bioluminescence levels. These unique selective pressures have had perhaps thousands of millions of years to sculpt the incredibly unique, diverse, and dynamic community inhabiting this realm of the deep sea (Nisbet and Sleep, 2001); Robison, 2004 suggests that members of this community may possess "biological adaptations and ecological mechanisms that we cannot yet imagine."

Since losing their protective external shell about 500 million years ago, cephalopods have comprised a significant portion of the mesopelagic community and continue to thrive, often times accounting for the majority of the predatory biomass in a given oceanic habitat (Rodhouse and Nigmatullin, 1996; Bush and Robison, 2007; Young, et al., 2012). Deep-sea cephalopods have other important roles in the food web of the mesopelagic zone, constituting a large portion of the diet of many pelagic fish, pinnipeds, cetaceans, seabirds, and other cephalopods (Croxall and Prince, 1996; Klages, 1996; Clarke, 1996; Smale, 1996; Dr. Henk-Jan Hoving pers. comm.). Historically, it has been predicted that the visual components of deep sea cephalopod behavior would be limited to transparency, silvering, red coloration, counter-illumination, and bioluminescence; they would not include complex behaviors similar to those utilized by their shallow-water counterparts for visual communication (Bush and Robison, 2007; Bush, et al., 2009). However, Bush and Robison, 2007 found that many deep sea teuthids produce six or more different ink release types, some of which have never been described in shallow-water cephalopods. Bush, et al., 2009 took it one step further by constructing a behavioral ethogram of the mesopelagic squid, *Octopoteuthis deletron*; they consequently described 59 unique chromatic, postural, locomotor, and bioluminescent behavioral components, comparable to or exceeding the number of unique behavioral components in ethograms constructed for shallow-water species of squid.

The possibility that most, if not all, mesopelagic teuthids have complex chromatic, postural, locomotor, and bioluminescent behavioral components and behavioral sequences is not as unbelievable as it might sound. Vision is a crucial component of the sensory systems of many mesopelagic squids and fishes, as demonstrated by their possession of highly developed and large eyes (Robison, 1999). A common misconception about the mesopelagic habitat is that it is completely devoid of light. However, in the upper reaches of the mesopelagic zone, about 1% of sunlight from the surface waters is still present, though very diffuse (Robison, 1999). Despite this challenge, many midwater fishes have the ability to distinguish daylight down to the very lowest depths of the mesopelagic zone (Robison, 1999). Such visually-cued predators in are known to forage by looking upwards toward the surface, discerning the faint silhouettes of their prey against the lighter surface waters (Robison, 1999). Their cephalopod prey, in contrast, are known to possess many camouflaging adaptations to avoid detection. In the upper reaches of the mesopelagic zone cephalopods have transparent bodies, silvering, and downward-directed bioluminescence to help break up their silhouettes (Robison, 1999; Herring, 2002). At depths around 600 m, cephalopod bodies become less transparent while increasing the number and density of brown, orange, and red body chromatophores (Robison, 1999; Herring, 2002). Many mesopelagic squids are also known to participate in diel migration, staying deeper during the day and returning to shallower depth during the night in order to avoid detection by visually-cued predators (Rodhouse and Nigmatullin, 1996; Robison, 2003).

If sunlight is only minimally present in the mesopelagic habitat, but has such a strong influence on behavior, then bioluminescence must have a huge influence on behavior as it is extremely prevalent in the midwater zone (Dr. Bruce Robison, pers. observ.). Herring, 2002 remarks that "all the uses to which light and color are put in the shallows, or on land, can be achieved in the dark environment of the deep sea by using bioluminescence." Bioluminescent light is not only produced in the aforementioned circumstance of defensive counter-shading by prey animals, but it is also produced by predators for the purpose of prey attraction and by many species for communicative purposes (Hunt, 1996; Herring, 2002; Robison, 2004). Additionally, the many small, planktonic light-producing fauna of the mesopelagic zone have the propensity to luminesce when disturbed, thus exposing animals in the near vicinity of the disturbance to visually-cued predators (Herring, 2002; Robison, 2004; Dr. Sean Lema, pers. comm.) The ambient light produced by the many bioluminescent organisms of the mesopelagic zone allows for the possibility of attack not only from below, but also from above or laterally (Dr. Bruce Robison, pers. comm.). Understandably, many behavioral adaptations have developed and proliferated in taxa of the mesopelagic zone in response to the high levels of bioluminescent light in this locality. These behaviors include low activity levels between crepuscular periods of diel migration, ambush and stealth predation strategies, and mimicry as a form of defense (Robison, 2004).

Cases of protective mimicry in the deep ocean have only been documented within the mesopelagic zone; they occur between depths of 200 and 800 m where light is limiting, but not absent (Robison, 1999; Herring 2002). In organismic mimicry systems, there are three participants: the *model*, the *mimic*, and the *dupe* (Pasteur, 1982). For protective mimicry to be successful in a given habitat, said habitat must contain an abundance of unpalatable models (Robison, 1999). For these reasons, the mesopelagic habitat contained within and offshore of Monterey Bay, California is a likely location to observe cases of protective mimicry. An overwhelming abundance and diversity of gelatinous fauna, including medusae, ctenophores, and salps, provide likely models in this locality; they all not only lack in significant nutrient density to make them worthwhile targets for most active predators, but the medusae also possess abundant stinging nematocysts (Robison, 1999). Not surprisingly, cases of medusa mimicry in two species of zoarcid fish, two species of chaetognaths, two species of tomopterids, and two species of appendicularians in this locality have been documented by Robison, 1999. Bush and Robison, 2007 also suggest that the ink ropes produced by the resident mesopelagic squid, *Gonatus onyx*, remarkably resembles various species of elongate siphonophores also populating this midwater habitat. In addition, short chains of colonial salps in this habitat often form spherical, medusa-like congregations as opposed to their extensive solitary or linear gatherings (Dr. Bruce Robison, pers. observ.).

Since the advent of deep sea observation and exploration utilizing remotely operated vehicles (hereafter ROVs) in Monterey Bay by the Monterey Bay Aquarium Research Institute (hereafter MBARI), one particular teuthid has been making a *dupe* out of scientists and ROV pilots alike; this is *Chiroteuthis calyx* (Young, 1972). *Chiroteuthis calyx* is a solitary, abundant mesopelagic squid unique to the temperate waters of the Northern Pacific (Roper and Young, 2013). It is commonly known as the swordtail squid, owing to the large ornamented extension of the gladius, or tail, that only the juvenile (Doratopsis) life stage possesses. An intact tail not only more than doubles the length of the animal, but it also gives the squid a remarkable resemblance to the siphonophore, *Nanomia bijuga* [an abundant and active predator inhabiting a very similar depth range (Robison, et al. 1998)]. It has long been hypothesized that juvenile *C. calyx* mimic this siphonophore to deter predation, but, aside from morphological comparisons, no other significant evaluations have been made (Vecchione, et al., 1992; Hunt, 1996; Seibel, et al., 2004; Bush and Robison, 2007; Roper and Young, 2013).

The juvenile's tail is not the only physical trait *C.* calyx is known for; it is also has many other intriguing morphological adaptations to life in the mesopelagic zone. Both the juvenile and subadult *C. calyx* life stages have four pairs of arms with pronounced arm keels; each successive arm pair is larger than the pair preceding it [1st pair is the smallest and 4th pair the largest] (Vecchione, 1992). Two elongate tentacles rest within sheathing grooves on the fourth arms that, when fully deployed, are almost twice the length of the entire animal (Vecchione, et al., 1992; Hunt, 1996). Photophores are found along the lengths of the tentacle stalks and fourth arms, dotting the tentacle clubs, and nestled on the ventral surface of the ink sac and eyes (Vecchione, et al., 1992; Hunt, 1996; Herring, 2002; Roper and Young, 2013); all can be illuminated or darkened by contracting or expanding the surrounding chromatophores, respectively (Vecchione, et al., 1992; Hunt, 1996). The dorsal surface of the animal is covered by chromatophores [with reduced coverage on the ventral and lateral surfaces] from tail tip to tentacle tips; they are arranged into distinct cohorts on the tail, mantle, neck, head, arms, and tentacles

(Hunt, 1996). All cohorts of chormatophores can be expanded or contracted individually or in unison (Vecchione, et al., 1992; Hunt, 1996; Bush and Robison, 2007).

Chiroteuthis calyx undergoes a tremendous morphological transformation as it ages (Hunt, 1996). The juvenile life stage is characterized by a pronounced and ornate tail, transparent and frail body tissue, an elongate neck and mantle, fewer chromatophores, and chromatophores of an orange-brown color (Vecchione, et al., 1992; Hunt, 1996; Robison, 2004). As the animal progresses into its subadult form, the tail is significantly shortened and/or eventually autotomized (Hunt, 1996), the body tissue becomes more opaque and sturdy (Robison, 2004; pers. observ.), the neck virtually disappears (Hunt, 1996; Bush and Robison, 2007), the mantle becomes proportionally much shorter (Hunt, 1996), the arms [especially the 4th pair] become proportionally longer and more robust (Hunt, 1996; Seibel, et al., 2004), the arm keels become more expansive (pers. observ.), and the chromatophores take on a darker shade of red and become more numerous (Vecchione, et al., 1992; pers. observ.). The presence of developed gonads would indicate sexual maturity and thus the adult life stage, but a sexually mature specimen has yet to be observed or captured (Dr. Henk-Jan Hoving, pers. comm.). Accompanying this dramatic morphological change with age is a change in habitat; *C. calyx* undergoes a vertical ontogenic migration, increasing its depth of residence as it increase in age (Hunt, 1996). *Chiroteuthis calyx* is also thought to undertake diel vertical migrations during the juvenile and subadult life stages (Hunt, 1996; Katugin and Zuev, 2007). Accompanying the morphological changes are many behavioral changes. However, little is known about the behavior of this cephalopod even though it is a very commonly encountered squid in the mesopelagic habitat of the Northern Pacific.

Very few behavioral studies have been conducted on mesopelagic squid (Bush, et al., 2009) and no thorough investigation of *C. calyx* behavior has been performed. Vecchione, et al. 1992 examined the behavior of the juvenile [Doratopsis] life stage using ROVs to observe the animals *in situ.* They noted that the juveniles can be found either vertically or horizontally in the water column, but have a typical behavioral response to the presence of the ROV of swimming down slowly tail-first with their body in line with the tail. They also noted a typical escape reaction, an inking pattern, and some ways that

C. calyx juveniles can darken their chromatophopres and illuminate their photophores. Hunt, 1996 used ROV and laboratory observations to describe the horizontal foraging behavior seen in both juvenile and subadult *C. calyx* known as the "fishing posture" as well as expanding upon the behaviors associated with the pseudomorph ink display. He also elaborated on juvenile and adult chormatophore and photophore use, describing a defensive bioluminescent behavior seen in subadults. Bush and Robison, 2007 used ROV observations to describe ink cloud use in subadults and ink pseudomorph series use in both age groups, noting that juveniles inked more frequently than subadults. They also mentioned some typical threat response patterns elucidated by subadults and juveniles when approached by the ROV.

Despite these advancements, a behavioral ethogram of *C. calyx*, and thus a means by which a significant comparison of juvenile and subadult behavior can be made, has yet to be completed. A comparison of this nature may offer an explanation for the drastic morphological differences between juveniles and subadults, potentially providing evidence for juvenile *C. calyx* mimicry of *N. bijuga.* In addition, while physiological comparisons have been made between *N. bijuga* and juvenile *C. calyx*, no behavioral comparison has been conducted between these two species. A behavioral comparison between them could offer additional evidence for the hypothesized case of *N. bijuga* mimicry by juvenile *C. calyx*.

MATERIALS AND METHODS

BEHAVIORAL ETHOGRAM OF *C. calyx*

To construct a behavioral ethogram of *C. calyx*, 78 members of this species (50 juveniles and 28 subadults) were observed *in situ* using over 6 hours of dive footage recorded from about 30 dives performed by three ROVs owned and operated by MBARI. The ROV *Tiburon*, which is no longer in use, was an electric vehicle capable of diving to 4000 m. It was equipped with a high definition (hereafter HD) camera and four 400-W DSPL HID Daylight lamps capable of producing illumination in the daylight range. The ROV *Ventana* is a 40-hp electro-hydraulic vehicle currently in use on MBARI's R/V *Rachel Carson* capable of attaining depths of 1850 m. It is outfitted with an Ikegama HD camera with a HA10X5.2 Fujinon Zoom Lens and six 400-W DSPL HID Daylight lamps. The ROV *Doc Ricketts* is a 75-shp electro-hydraulic vehicle currently in use on MBARI's R/V *Western Flyer* capable of diving to a maximum depth of 4000 m. It is equipped with an Insite HDTV camera with 10x zoom and six 400-W DSPL HID Daylight lamps. Scientific cameras on these vehicles are operated by scientists and ROV pilots who have control over the camera's position, focus, zoom, aperture, and lighting. At the beginning of every dive, the recording camera is given a white balance to ensure accurate footage coloration. All footage during ROV dives is recorded on Panasonic D5 HD videocassettes and/or Sony Digital Betacam standard definition (herefter SD) videocassettes, contributing to the collection of over 18,000 hours of ROV footage taken from the past 23 years in the Monterey Submarine Canyon and surrounding waters by MBARI. Captured film is annotated by scientists and video-lab staff who record all of the organisms encountered during the dives using MBARI-designed Video Annotation Reference System software (hereafter VARS); these annotations are synchronized with hydrographic parameters to ensure maximum utility (Bush, et al., 2009).

Dive footage was viewed directly from ROV dive tapes on a Sony HR Trinitron monitor or a Sony MEU-WX2 Multiformat Engine and LMD-232W LCD monitor. Monitor settings maintained the accurate coloration established at the beginning of each dive. All recordings of *C. calyx* selected for observation were examined many times at normal to slow playback speeds to ensure accurate behavior description and identification. Similar to the methodology by which Bush, et al., 2009 created a behavioral ethogram for *O. deletron*, behaviors of *C. calyx* were subdivided into four categories of behavioral components: postural, chromatic, bioluminescent, and locomotor. These components were then used to describe several behavioral sequences commonly performed by *C. calyx*. For every individual, all behavioral components and sequences performed during the entire encounter were recorded as present or absent and then compiled into one of two groups, juveniles or subadults. The approximate life stage of an individual determined which cohort it fell into. Juveniles were identified by their possession of all or some of the tail and subadults were characterized by having no tail, or only very minute remnants of the proximal base of the tail. The resulting data table (*Table 1*) was then used to calculate the percent of juveniles and subadults performing each behavioral component or sequence in order to examine discrepancies in subadult and juvenile *C. calyx* behavior. Our null hypothesis was that juveniles and subadults would show the same suite and incidence of behavioral components across all four categories (i.e., they were from the same population). The alternative hypothesis was that juveniles and subadults would exhibit a different suite and incidence of behavioral components across all four categories (i.e., they were from different populations). Each of the four categories of components was tested against the null hypothesis by randomly resampling the data with replacement, or bootstrapping, 1000 times. For every new random sampling, a mean value was calculated and scored as equal to or greater than 2.5% or less than or equal to 97.5%. If the resampled values fell outside of that range less than 5% of the time ($p < 0.05$), the null hypothesis was rejected. Trends were noted in the behavioral sequence data, but no statistics were performed.

In situ observations of five juvenile *C. calyx* obtained on three R/V *Rachel Carson* expeditions, while operating the ROV *Ventana*'s HD camera, aided in identifying and describing the behavioral sequences and components presented in the following ethogram. Also, *in situ* experiments of removing the juvenile's tail via contact by the ROV aided in identifying physiological aspects of the tail, as well as behavioral changes by the juveniles in reaction to losing their tail. None of the squid observed by this method were included in the data presented in the behavioral ethogram. Also aiding in the identification and descriptions of behavioral sequences and components presented in the following ethogram, were lab observations of three juvenile *C. calyx* captured on R/V *Rachel Carson* expeditions by the ROV *Ventana* and her pilots. These specimens were kept alive in a dark, refrigerated room maintained at 5° Celsius in tanks designed and built by MBARI engineers and scientists specifically for midwater organisms. Specimens were observed under red lighting with naked eyes, with minimal fluorescent lighting and night vision goggles, and under infrared lighting with a Canon XL1 digital video camcorder equipped with an AstroScope night vision module. The latter of these techniques proved to be the most successful method of lab observation. While these individuals were not used as part of the data presented in the ethogram, they did exhibit some novel behavioral sequences.

ORIENTATION STUDY OF *C. calyx* AND *N. bijuga*

To conduct a study comparing the body orientation of *C. calyx* and *N. bijuga*, 42 juvenile *C. calyx* and 21 subadult *C. calyx* (both also composing part of the ethogram) were observed from the footage of 16 ROV *Doc Ricketts* dives from the fall of 2012, 2011, and 2010. Also 3,655 *N. bijuga* were observed from the footage of four ROV *Doc Ricketts* dives from the fall of 2012. All of these animals were observed on the same equipment used for constructing the behavioral ethogram and via the same methods. However, only the depth (m), time of day, initial tail orientation (up, down, or indeterminate), and reaction tail orientation (up, down, or indeterminate) were noted for *C. calyx* juveniles and subadults in this study. The initial tail orientation was the approximate direction the tail, or distal tip of the mantle if subadult, was pointed when an individual *C. calyx* was first encountered by the ROV. The reaction tail orientation is the approximate direction the individual *C. calyx* pointed its tail in response to the increased proximity or extended presence of the ROV. For every individual *N. bijuga*, the depth range (0-100, 100-200, 200-300, 300-400, 400-500, 500-600, or 600+ m), time range (06:30-09:30 or 13:00-18:00), and siphosome orientation (up, down, or indeterminate) were recorded. These recordings were then compiled into a table displaying the percent of *N. bijuga* in the three different orientations across the aforementioned depth ranges from 06:30 to 09:30 and 13:00 to 18:00 (*Table 2*). *Nanomia bijuga* and *C. calyx* were examined from only recent fall dives of the ROV *Doc Ricketts* because fall is the season that *N. bijuga* are known to be most abundant in Monterey Bay (Robison, et al., 1998). Only ROV *Doc Ricketts* dives were observed because this ROV was launched earlier in the morning and retrieved later in the evening than other MBARI-owned and managed ROVs. It was important that we examined the orientation of *N. bijuga* as early and late as possible in hopes of recording differences in their orientation across depths during periods of their known vertical migration. Because of the limited sample size of juvenile and subadult *C.calyx* that fell within the time ranges the *N. bijuga* were surveyed (n=25 for juveniles and 16 for adults), no significant statistical comparison could be made. Instead, trends were graphically displayed.

RESULTS

BEHAVIORAL ETHOGRAM OF *C. calyx*

78 individuals were observed: 50 juveniles and 28 subadults. The number of juveniles and subadults observed performing each behavioral component or sequence are indicated by the first and second numbers, respectively, and contained within the parentheses immediately following the name of the behavioral component or sequence. The behavioral components have been subdivided into four categories: postural, chromatic, bioluminescent, and locomotor; the total number of components within each subdivision is indicated by the number in parentheses immediately following the headings denoting each of these subdivisions. Behavioral sequences have their own subsection at the end of the ethogram with the same specifications as the aforementioned components. Behavioral sequences are described using behavioral components.

Postural components (21)

Tail down (39, 14): The tail or posterior mantle tip is pointed down and the animal is oriented in an oblique vertical position. This is a posture also seen in other squids of the genus *Octopoteuthis* (Bush, et al., 2009).

Tail up (12, 15): The tail or posterior mantle tip is pointed up and the animal is oriented in an oblique vertical position. This posture is also seen in *O. deletron* and various mastigoteuthids (Bush, et al., 2009).

Horizontal (25, 19): The squid's dorsal mantle is up and the animal remains horizontal in the water column. This is a posture also seen in many other squids, including the genus *Octopoteuthis* (Bush, et al., 2009).

Tentacles extended: Both of the feeding tentacle clubs and much of the tentacle stalks are released beyond the grooves of the sheathing fourth arms, often hanging beneath the arms or drifting behind the arms as the animal swims tail first. Tentacles can be either a) **fully** (32, 18) extended or b) **partially** (8, 5) extended. This posture has been noted previously in this species by Vecchione, et al., 1992.

Tentacles retracted (14, 18): Both of the feeding tentacle stalks and much, if not all of the tentacle clubs are completely held within the tentacle-sheathing, fourth arm grooves. This posture has been noted previously in this species by Vecchione, et al., 1992.

Tentacles together (9, 0): While tentacles are extended, they align in a straight line with the mantle and tail while touching along their entire length. This posture has been noted previously in this species by Vecchione, et al., 1992.

Tentacles spread: While tentacles are extended, they remain separated along their entire length. In this posture, the tentacles are a) **relaxed** (25, 12), or straight and flaccid while spread or b) **cupped** (8, 0), so that the distal portion of the tentacles curves outwards and then inwards; this almost points the distal tips of the clubs at each other with the space they encompass resembling an oval shape. This posture (a) has been noted previously in this species by Vecchione, et al., 1992.

Arms spread (40, 25): Each of the four pairs of arms is oriented in such a way that each arm of a pair angles away from the other arm of the pair while touching only proximally at their base, making a "v" or "u" shape. The first pair sits atop the second, the second above the third, and the third over the fourth. In this posture, each pair of arms can be in its own horizontal plane, or the first three pairs of arms can be spread in the same horizontal plane, with the fourth pair immediately below the first three. This posture has been noted previously in this species by Vecchione, et al., 1992. Bush, et al. 2009 described a very similar posture called "splayed arms" and "central arm space" in *O. deletron*.

Arms together: Arms are held together across their entire length in line with body, thus producing a tapering point from their attachment to their distal tips. Either a) **all of the arms** (26, 13) are held together across their entire length, or b) **all arms except for the fourth** (1, 0) pair are held together while the fourth pair is spread to a minimal, but varying degree. This posture has been noted previously in this species by Vecchione, et al., 1992 and Bush, et al., 2009 described a very similar posture called "straight arms" in *O. deletron*.

Arm keels prominent: Arms have their keels stretched to their full width, thus maximizing the surface area of the animal. This can occur where either a) **all pairs** (20,

20) of arms have prominent keels, b) **only the third and fourth pairs** (0, 1) of arms have prominent keels, c) **only the third pair** (3, 2) of arms has prominent keels, or d) **only the fourth pair** (1, 0) of arms has prominent keels.

Arm tips curled: While arms are spread or together, the tips of the arms curve inwards toward the buccal cavity of the squid. This can occur where either a) **all pairs** (40, 21) of arms are curled, b) **only the third and fourth pairs** (0, 1) of arms are curled, or c) **only the third pair** (4, 1) of arms is curled. This posture (a) has been noted previously by Bush and Robison, 2007 and is often co-occurs with the postural component "all arm keels prominent." Bush, et al., 2009 described a very similar posture called "arms curled" in *O. deletron*.

Tail expanded (8, 0): Tissue attached to extended gladius of tail is stretched to its full potential width, making the tail wider in appearance. This postural component is unique to juveniles.

Tail contracted (1, 0): Tissue attached to extended gladius of tail is constricted to its minimal width, making the tail narrower in appearance. This postural component is unique to juveniles.

Chromatic components (26)

Pale (27, 13): No chromatophores on the entire animal are expanded, resulting in a mostly transparent appearance in juveniles and young subadults, and an opaque appearance in older subadults. "Pale" is a chromatic component previously described by Bush, et al., 2009 in the species *O. deletron*. Many other genera of cephalopods, including *Doryteuthis, Loligo, Sepioteuthis, Alloteuthis, Sepiola, Gonatus,* and *Mastigoteuthis* have similar chromatic behaviors (Bush. et al., 2009)

Dark (28, 8): All chromatophores on the entire animal are expanded, thus making the squid appear to be mostly brown orange (if juvenile) to dark red (if subadult). This chromatic behavioral component has been previously described in this species by Vecchione, et al., 1992 and Hunt, 1996. Bush, et al., 2009 also described a similar chromatic behavioral component in *O. deletron* with the same name and mentioned that it occurs in the genera *Doryteuthis, Loligo, Sepioteuthis, Alloteuthis, Sepiola, Gonatus,* and *Mastigoteuthis.*

Dark dorsal mantle (11, 5): Chromatophores along dorsal midline of the mantle are expanded, thus creating a thick, brown-orange to dark red stripe running from the posterior tip to the anterior edge of the mantle and above the internal digestive gland, organs, and gills. This chromatic behavioral component has been previously noted in this species by Vecchione, et al., 1992; Hunt, 1996, and Bush and Robison, 2007.

Dark ventral mantle (7, 2): Chromatophores along the ventral midline of the mantle are expanded, thus creating a brown-orange to dark red patch below the internal digestive gland, organs, and gills. Vecchione, et al., 1992 and Hunt, 1996 have previously noted patches of chromatophores on the mantle in this vicinity.

Dark lateral mantle (7, 2): Chromatophores on lateral surface of the mantle are expanded, thus creating a brown-orange to dark red patch in the gill region at the anterior end of the mantle with scattering to the posterior end. The presence of chromatophores in this vicinity has been previously noted by Vecchione, et al., 1992.

Dark mantle tip (10, 3): Chromatophores at the tip of the mantle that extend out into the distal portions of the fins nearest to the tail are expanded, thus creating a brown-orange to dark red patch in the this location. The presence of chromatophores in this vicinity has been previously noted by Vecchione, et al., 1992. This chromatic behavior is roughly the opposite of what Bush, et al., 2009 described as “pale tail” in *O. deletron*.

Dark dorsal neck (11, 1): Chromatophores along dorsal midline of the neck are expanded, thus creating a thick, brown-orange to dark red stripe running from the posterior to anterior end of the neck. This chromatic behavior has been previously noted in this species by Vecchione, et al., 1992; Hunt, 1996, and Bush and Robison, 2007.

Dark throat (8, 1): Chromatophores on the ventral surface of head from the arm crown pillar to directly beneath the eyes are expanded, thus creating a brown-orange to dark red stripe in this location. The presence of chromatophores in this vicinity has been previously noted by Vecchione, et al., 1992.

Dark tail midline (15, 0): The speckled pattern of chromatophores on the majority of the tail tissue is expanded, thus changing the majority of the tail from transparent to brown-orange or orange. This chromatic behavior has been previously noted in this species by Vecchione, et al., 1992 and Hunt, 1996. This chromatic component is unique to juveniles.

Dark tail pouches (32, 0): Chromatophores covering the 3-6 pairs of pouches running down the length of the tail are expanded, thus turning the pouches from transparent to brown-orange or orange. This chromatic behavior has been previously noted in this species by Vecchione, et al., 1992 and Hunt, 1996. This chromatic component is unique to juveniles.

Dark tail tip (13, 0): Chromatophores on the tissue of the tapering distal tip of the tail are expanded, thus turning the distal, tapering end of the tail from transparent to brown-orange or orange. This chromatic component is unique to juveniles.

Dark tail base (13, 0): Chromatophores on the tissue of the constricted, proximal base of the tail are expanded, thus turning the proximal, constricted base of the tail from transparent to brown-orange to orange. This chromatic component is unique to juveniles.

Dark fins edges (8, 6): Thin, tapering, and arched stripes of chromatophores on the outside edges of the ventral surfaces of both fins are expanded, thus darkening this area a brown-orange to dark red. This chromatic behavior has been previously noted in this species by Vecchione, et al., 1992 and Hunt, 1996. This is almost opposite to the behavior "pale fin edge" that Bush, et al., 2009 described for the species *O. deletron*.

Dark fin centers (8, 5): Patches of chromatophores on the center of the dorsal surface of the fins are expanded, thus darkening this area a brown-orange to dark red. This chromatic behavior has been previously noted in this species by Vecchione, et al., 1992 and Hunt, 1996. This is almost opposite to the behavior "pale lateral fin stripe" that Bush, et al., 2009 described for the species *O. deletron*.

Dark funnel (9, 2): Chromatophores on and surrounding the funnel and collar are expanded, thus coloring these structures brown-orange to dark red. This chromatic behavior has been previously noted by Hunt, 1996.

Dark eyebrow patches (12, 6): Chromatophores running along the dorsal midline of the head, but most concentrated between the eyes, are expanded, thus creating a brown-orange to dark red patch in this area. This chromatic behavior has been previously noted in this species by Vecchione, et al., 1992; Hunt, 1996, and Bush and Robison, 2007 and also noted in *G. onyx, G. phyllura*, and *Sepioteuthis australis* (Bush, et al., 2009).

Dark eye circle (4, 2): The narrow bands of chromatophores encompassing the eyes are expanded, thus creating a brown-orange to dark red circle around the eyes. This posture has been previously noted in this species by Bush and Robison, 2007. Bush, et al., 2009 also described a similar chromatic behavior in *O. deletron*.

Dark Arms (10, 7): Chromatophores on the aboral surface of the arms are expanded, thus coloring the dorsal surface of all arms a brown-orange to dark red. This chromatic behavior has been previously noted in this species by Vecchione, et al., 1992; Hunt, 1996, and Bush and Robison, 2007. It is also similar to what Bush, et al., 2009 described as “dark aboral arms” and “dark oral arms” in the species *O. deletron*.

Dark Arm tips: Chromatophores at the distal tips of the arms on the aboral surface are expanded, thus coloring the arm tips a brown-orange to dark red. The proximal end of the coloration on the arm tips during this chromatic component ends rather abruptly in a straight line. This can occur in a) **all four pairs** (13, 12) of arms, b) **only the first three pairs** (5, 0) of arms, c) **only the third and fourth pairs** (2, 6) of arms, d) **only the third** (1, 3), or e) **only the fourth pair** (2, 5) of arms.

Dark arm band (2, 1)**:** Chromatophores on the aboral surface of the fourth arms, from the distal tip of the tentacular sheath down to about one quarter of the way down the tentacular sheath in the anterior direction, are expanded. This creates a brown-orange to dark red band with abrupt, linear edges on both its anterior and posterior sides.

Dark tentacles (1,1): Lines of chromatophores on the aboral surface of the tentacle clubs and stalks are expanded, creating a brown-orange to dark red color that extends from the distal tip of the club down the to the proximal attachment of the stalk. The presence of chromatophores in this vicinity has been previously noted by Vecchione, et al., 1992.

Dark tentacle tips (18, 2): Small and concentrated patches of chromatophores on the aboral surface of the outside, distal potion of the tentacle clubs expand to create dark orange to red patches. The presence of chromatophores in this vicinity has been previously noted by Vecchione, et al., 1992.

Bioluminescent components (5)

Ocular photophores illuminated (50, 28): Two photophores on the reflective tissue of the ventral surface of each eye are continually exposed (Hunt, 1996; Roper and Young, 2013). The eyes rotate to keep the ocular photophores pointed down when the body orientation changes, indicating the function of counter-illumination (Hunt, 1996).

Visceral photophores illuminated (50, 28): Two photophores on the ventral side of the ink sac and digestive gland are continually exposed (Roper and Young, 2013). The digestive gland and ink sac rotate to keep the visceral photophores pointed down when the body orientation changes, indicating the function of counter-illumination (Hunt, 1996).

Tentacle club photophores illuminated (16, 8): Photophores on the oral and aboral surfaces of the tentacle clubs are exposed when their concealing chromatophores are retracted, thus illuminating the tentacle clubs. This bioluminescent behavior has been described in this species by Hunt, 1996.

Tentacle stalk photophores illuminated (6, 6): Chromatophores concealing each of the serial photophores that run the length of the tentacle stalks are retracted, exposing a row of light organs that run from the base of the club to the arm crown pillar. This bioluminescent behavior has been described in this species by Hunt, 1996.

Fourth arm photophores illuminated (1, 5): Chromatophores concealing each of the serial photophores that run the length of the fourth arms are retracted, exposing a row of light organs that runs from the arm tip to base. Photophores in this vicinity have been noted by Roper and Young, 2013.

Locomotor components (21)

Tail autotomy: The ornate extension of the gladius present on juveniles is either broken off from physical stress, or released from the animal's body. This occurs as a last resort escape mechanism when the animal is under a great deal of stress (Bush, 2012). The gladius can break a) **partially** (3, 0) at one of a multitude of points along the tail, or b) **entirely** (8, 0) at the proximal end of the tail where it attaches to the mantle. This locomotor behavior has been previously described by Vecchione, et al., 1992 and Hunt, 1996. Autotomy is not unique to *C. calyx*, as arm autotomy has been described in *O. deletron*, *O. megaptera*, *O. neilseni*, and *Vampiroteuthis infernalis* (Bush, et al., 2009; Bush, 2012).

Tail expansion (1, 0): The process whereby the tissue attached to the extension of the gladius is stretched to its full potential width, thus making the tail wider in appearance. This locomotor component is unique to juveniles.

Tail contraction (1, 0): The process whereby the tissue attached to the extension of the gladius is constricted to its minimum potential width, thus making the tail narrower in appearance. This locomotor component is unique to juveniles.

Tentacle retraction (13, 8): Transverse and circular muscle fibers of the tentacles are constricted (Kier, 1982), shortening the stalk and thus transporting the clubs towards the body and into the tentacle-sheathing grooves of the fourth arms. The tentacles can be constricted slowly or rapidly and the retraction can be complete or partial. Vecchione, et al., 1992; Hunt, 1996; Robison, 2004, and Bush and Robison, 2007 have all previously noted this locomotor behavior. Many cephalopods, including members of the family Loliginidae, extend and retract their tentacles (Kier, 1982).

Tentacle extension (8, 6): Longitudinal muscle fibers of the tentacles are constricted (Kier, 1982), thus extending the tentacles from the sheathing fourth arm grooves. The tentacles can be extended slowly or rapidly and the extension can be complete or partial. Vecchione, et al., 1992; Hunt, 1996; Robison, 2004, and Bush and Robison, 2007 have all previously noted this locomotor behavior in this species. Many cephalopods, including members of the family Loliginidae, extend and retract their tentacles (Kier, 1982).

Tentacle spread (4, 7): During or after tentacle extension, the fourth arms are used to spread the tentacles laterally and away from the body. This locomotor behavior has been previously described in this species by Hunt, 1996.

Fin undulation (50, 28): The fins are moved up and down around the mantle, curling dorsally on the upstroke and curling ventrally on the downstroke. Waves are created that run along the length of the fins and slowly propel the squid either forward (arms-first), backwards (tail first), or enable it to remain stationary in the water column. This locomotor behavior has been noted by Vecchione, et al., 1992 in this species. "Fins flapping" is a similar behavior described by Bush, et al., 2009 in *O. deletron*. Analogous locomotor behaviors also occur in *M. magna*, *O. megaptera*, and *Taningia danae* (Bush, et al., 2009).

Backward jetting (22, 2): Discharges from the funnel, coupled with rapid backward fin undulation, are used to propel the squid tail-first at speeds up to 3 body lengths per second. This locomotor behavior has been noted by Vecchione, et al., 1992 in this species. A similar behavior of the same name has been described in *O. deletron* by Bush, et al., 2009.

Forward jetting (12, 3): Discharges from the funnel, coupled with rapid forward fin undulation, are used to propel the squid arms-first at speeds up to 3 body lengths per second. A similar behavior of the same name has been described in *O. deletron* by Bush, et al., 2009.

Backward swimming (35, 10): Funnel discharges and/or backward fin undulations are used to propel the animal tail-first in any direction. A similar behavior with the same name was described by Bush, et al., 2009 in the species *O. deletron*.

Forward swimming (5, 6): When funnel discharges and/or forward fin undulations are used to propel the squid arms-first in any direction. A similar behavior with the same name was described by Bush, et al., 2009 in the species *O. deletron*.

Jolt (0, 2): A sudden and rapid convulsion, or series of convulsions, moving the whole body. Jolts were only observed when the animals were severely agitated; they would typically occur in the middle of a behavior sequence or would signal the transition into a new behavior sequence. Jolts are vastly different from the more common, fluid movements that this animal is normally observed performing. A similar behavior with the same name was described by Bush, et al., 2009 in the species *O. deletron*.

Direction Change (12, 3): A sudden alternation between forward and backward swimming or forward and backward jetting where the animal will abruptly propel itself in the opposite direction and with the opposite body orientation that it, until that instance, demonstrated. This locomotor behavior occurred when animal had been touched by the ROV or perused for a great deal of time.

Hovering (36, 27): Minimal fin undulation and various body postures are used to keep the animal stationary in the water column; while hovering, the animal could be oriented head-up, head down, or horizontal. This behavior has been previously described in *O. deletron*, *O. megaptera*, and many other cephalopods (Bush, et al., 2009).

Grasp (1, 0): While making contact with the ROV, the animal wraps its tentacles around the surface of the vehicle, namely the suction sampler nozzle. This locomotor behavior was usually followed by backward jetting. Bush, et al., 2009 described a similar behavior with the same name for the species *O. deletron*.

Ink release: Ink was ejected from the ink gland; inking animals could be observed releasing several different forms. The first form is a **pseudomorph** (21, 1), or thin, sometimes wavering string of ink approximately the same length and diameter as the animal that produced it (Vecchione, et al., 1992; Bush and Robison, 2007). Pseudomorphs are often associated with hovering or jetting (Hunt, 1996); they are thought to provide a visually oriented predator with an alternate target, or to mimic the siphonophore *Nanomia bijuga* (Bush and Robison, 2007). The second form is a long **series of pseudomorphs** (3, 0) that overlap to create a very long, twisting strand of ink roughly the same width, but many times the length of the animal that produced it. This form is also associated with hovering or jetting and could potentially mimic elongate siphonophores (Bush and Robison, 2007), as they were often nearby when this ink release occurred. The third form is a **cloud** (2, 11), or diffuse layer of ink directly above or next to the individual that released it; clouds are associated with hovering and little movement, perhaps acting as a device of concealment (Bush and Robison, 2007). The fourth form is a **rope** (2, 0), or a long, thin string of ink punctuated by occasional, small splotches of thicker ink. Ropes could also imitate species of elongate, but thinner siphonophores (pers observ.); they were often nearby when this ink release occurred. The fifth and final ink form is a **series of puffs** (2, 0), occurring when an individual would sequentially release small, spherical puffs of ink in all directions, creating a scattering of small clouds surrounding the animal. This form is associated with hovering.

Common behavioral sequences (5)

Fishing (14, 21)**:** Horizontal or tail up, hovering, fin undulation, all arms spread, all arm tips curled or not, all arm keels prominent, tentacles fully extended to retracted, pale or arm tips dark, ocular photophores illuminated, visceral photophores illuminated, tentacle club photophores illuminated or not, tentacle stalk photophores illuminated or not, and ink cloud present or absent. This behavioral sequence has been described by Hunt, 1996 and Robison, 2004.

Spar buoy (18, 1)**:** Tail down, hovering, fin undulation, all arms spread, all arm tips curled or not, all arm keels prominent or not, tentacles fully to partially extended, pale or dark tail pouches, ocular photophores illuminated, visceral photophores illuminated, tentacle club photophores illuminated or not, and tentacle stalk photophores illuminated or not.

Swimming down tail first (36, 7)**:** Tail down, backward swimming, fin undulation, all arms together or all arms spread, tentacles extended to partially extended, 3rd pair arm tips curled or not, 3rd pair arm keels prominent or not, dark tail pouches or not, only 3rd pair arm tips dark or not, only 3rd and 4th pair arm tips dark or not, all arm tips dark or not, ocular photophores illuminated, visceral photophores illuminated, and tentacle club photophores illuminated or not. Squid progressively expanded additional chromatophores, thus turning more brown-orange to dark red as our observation time increased. A similar behavioral sequence has been described by Vecchione, et al., 1992.

Tail down hover (14, 1)**:** Tail down, hovering, fin undulation, tail expanded or not, all arms spread, all arm tips curled, all arm keels prominent, tentacles fully to partially extended, tentacles together or not, tentacles spread and cupped or not, dark, ocular photophores illuminated, and visceral photophores illuminated.

Dark when touched (15, 1): Tail up, down, or horizontal, forward or backward jetting, forward or backward swimming, all arms together, tentacle retraction or not, tentacles retracted to partially extended, pseudomorph ink release, partial tail autotomy or not, entire tail autotomy or not, and dark. A similar behavioral sequence has been described by Vecchione, et al., 1992.

Postural components (21)	**# Juveniles**	**# Subadults**	**% Juveniles**	**% Subadults**
Arms spread	40	25	80	89.28571429
All arm tips curled	40	21	80	75
Tail down	39	14	78	50
Tentacles fully extended	32	18	64	64.28571429
All arms together	26	13	52	46.42857143
horizontal	25	19	50	67.85714286
Tentacles spread and relaxed	25	12	50	42.85714286
All arm keels prominent	20	20	40	71.42857143
Tentacles retracted	14	18	28	64.28571429
Tail up	12	15	24	53.57142857
Tentacles together	9	0	18	0
Tentacles partially extended	8	5	16	17.85714286
Tentacles spread and cupped	8	0	16	0
Tail expanded	8	0	16	0
Only 3rd pair arm tips curled	4	1	8	3.571428571
Only 3rd pair arm keels prominent	3	2	6	7.142857143
All arms except fourth pair together	1	0	2	0
Only 4th pair arm keels prominent	1	0	2	0
Tail contracted	1	0	2	0
Only 3rd and 4th pair arm keels prominent	0	1	0	3.571428571
Only 3rd and 4th pair arm tips curled	0	1	0	3.571428571

Chromatic components (26)	**# Juveniles**	**# Subadults**	**% Juveniles**	**% Subadults**
Dark tail pouches	32	0	64	0
Dark	28	8	56	28.57142857
Pale	27	13	54	46.42857143
Dark tentacle tips	18	2	36	7.142857143
Dark tail midline	15	0	30	0
Dark tail tip	13	0	26	0

Dark tail base	13	0	26	0
All arm tips dark	13	12	26	42.85714286
Dark eyebrow patches	12	6	24	21.42857143
Dark dorsal mantle	11	5	22	17.85714286
Dark dorsal neck	11	1	22	3.571428571
Dark mantle tip	10	3	20	10.71428571
Dark arms	10	7	20	25
Dark funnel	9	2	18	7.142857143
Dark throat	8	1	16	3.571428571
Dark fin edges	8	6	16	21.42857143
Dark fin centers	8	5	16	17.85714286
Dark ventral mantle	7	2	14	7.142857143
Dark lateral mantle	7	2	14	7.142857143
Only pairs 1-3 arm tips dark	5	0	10	0
Dark eye circle	4	2	8	7.142857143
Only 3rd and 4th pair arm tips dark	2	6	4	21.42857143
Only 4th pair arm tips dark	2	5	4	17.85714286
Dark arm band	2	1	4	3.571428571
Only 3rd pair arm tips dark	1	3	2	10.71428571
Dark tentacles	1	1	2	3.571428571
Bioluminescent components (5)	**# Juveniles**	**# Subadults**	**% Juveniles**	**% Subadults**
Ocular photophores illuminated	50	28	100	100
Visceral photophores illuminated	50	28	100	100
Tentacle club photophores illuminated	16	8	32	28.57142857
Tentacle stalk photophores illuminated	6	6	12	21.42857143
Fourth arm photophores illuminated	1	5	2	17.85714286
Locomotor components (21)	**# Juveniles**	**# Subadults**	**% Juveniles**	**% Subadults**
Fin undulation	50	28	100	100
Backward swimming	36	10	72	35.71428571
Hovering	36	27	72	96.42857143
Backward jetting	22	2	44	7.142857143
Pseudomorph ink release	21	1	42	3.571428571
Tentacle retraction	13	8	26	28.57142857
Forward jetting	12	3	24	10.71428571
Direction change	12	3	24	10.71428571
Entire tail autotomy	8	0	16	0
Tentacle extension	8	6	16	21.42857143
Forward swimming	5	6	10	21.42857143
Tentacle spread	4	7	8	25
Partial tail autotomy	3	0	6	0

Pseudomorph series ink release	3	0	6	0
Cloud ink release	2	11	4	39.28571429
Rope ink release	2	0	4	0
Series of puffs ink release	2	0	4	0
Tail expansion	1	0	2	0
Tail contraction	1	0	2	0
Grasp	1	0	2	0
Jolt	0	2	0	7.142857143
Behavioral sequences (5)	**# Juveniles**	**# Subadults**	**% Juveniles**	**% Subadults**
Swim down tail first	36	7	72	25
Spar buoy	18	1	36	3.571428571
Dark when touched	15	1	30	3.571428571
Fishing posture	14	21	28	75
Tail down hover	14	1	28	3.571428571

Table 1: Number and percent of juvenile and subadult *C. calyx* seen performing each behavioral component or sequence. All behavioral components and sequences are ranked within their respective sections in descending order by percent of juveniles performing behavior components or sequences.

06:30-09:30			
Depth range (m)	**% siphosome down**	**% siphosome up**	**% indeterminate**
0-100	8.333333333	68.00595238	23.66071429
101-200	7.175925926	75.11574074	17.70833333
201-300	15.03161127	66.73697903	18.2314097
301-400	66.66826672	19.09645786	14.23527542
401-500	72.78639144	15.38953491	11.82407364
501-600	91.0962911	1.766701767	7.137007137
600+	0	0	0
13:00-18:00			
Depth range (m)	**% siphosome down**	**% siphosome up**	**% indeterminate**
0-100	40	0	60
101-200	16.81693989	12.85915163	36.57103825
201-300	35.90717838	17.7234678	46.65306643
301-400	50.50961117	22.20567166	30.94867346
401-500	63.71022232	13.2606052	26.0081936
501-600	78.92487629	2.773303712	17.78927375
600+	95.23809524	0.952380952	3.80952381

Table 2: The percent of *N. bijuga* oriented siphosome down, up, and indeterminately across depth ranges in the morning (06:30-09:30) and in the evening (13:00-18:00). This table is visually represented in *Figure 1*.

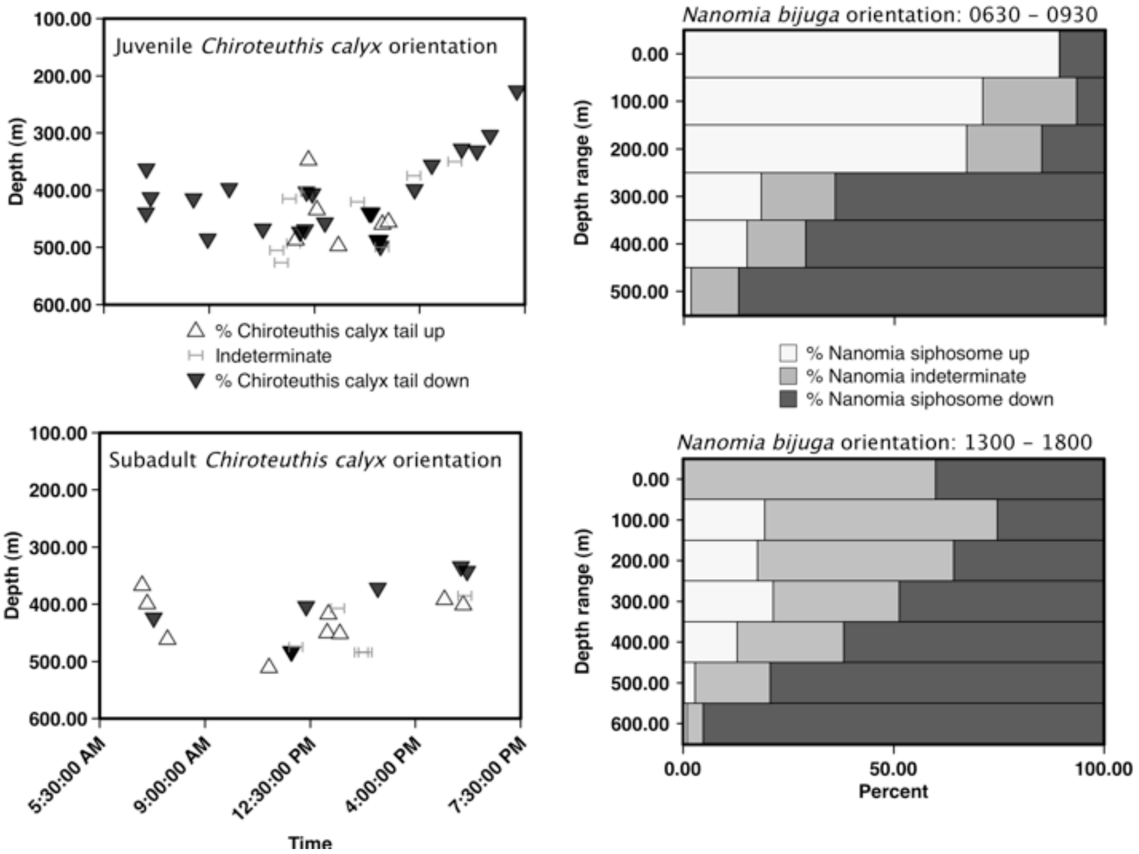

Figure 1: As indicated by the figure in the upper right, *N. bijuga* examined in the morning (06:30 to 09:30) were mostly be oriented siphosome up above 300 m in depth (68% in the 0-100 m range, 75% in the 101-200 m range, and 67% in the 201-300 m range) and siphosome down below 300 m (67% in the 301-400 m range, 73% in the 401-500 m range, and 91% in the 501-600 m range). As indicated by the figure in the lower right, *N. bijuga* observed in the evening (13:00 to 18:00) were found to mainly orient siphosome down below 300 m in depth (51% in the 301-400 m range, 64% in the 401-500 m depth, and 79% in the 501-600 m range), but mostly oriented indeterminately above 300 m (60% in the 0-100 m range, 37% in the 101-200 m range, and 47% in the 201-300 m range). As indicated by the figure in the upper left, *C. calyx* juveniles were found to mostly orient tail down below 300 m in depth. As indicated by the figure in the lower left, subadult *C. calyx* were equally distributed across the three orientations below 300 m.

JUVENILE AND SUBADULT *C. calyx* BEHAVIORAL COMPARISON

Randomly resampling (bootstrapping) the data 1000 times with an acceptance level of 5% revealed that the behaviors of subadult and juvenile *C. calyx* differed significantly (i.e., they were from different populations). While we found no significant difference in the chromatic and locomotor components of juveniles and subadults, we did find a significant difference in the postural and bioluminescent components of juveniles and subadults ($p < 0.05$). For the 5 behavioral sequences described, some interesting trends were noted. Three of the 4 behavioral sequences more commonly performed by juveniles than subadults (Swim down tail first [72% of juveniles, 32% of subadults], spar buoy [36% of juveniles, 3.57% of subadults], and tail down hover [28% of juveniles, 3.57% of subadults]) incorporated a tail down orientation. The one behavioral sequence more commonly performed by subadults than juveniles (fishing posture [75% of subadults, 28% of juveniles]) involved a horizontal or indeterminate orientation.

A juvenile *C. calyx*, classified by its possession of all or some of the tail, was typically pale when encountered, its tail either down in the spar buoy posture, or up or horizontal in a posture similar to the subadult fishing posture, but with the arms and head angled up more. When alarmed, the juvenile immediately oriented its tail down and began slowly swimming down while simultaneously darkening its tail pouches. When the juvenile became more alarmed, it darkened specific regions around its photophores and around opaque organs and began to curl its 3rd and/or 4th pair of arms. When it became even more disturbed, it stopped swimming down and hovered with minimal fin undulation while oriented tail-down. It then became dark while spreading all arms, curled the tips of all arms, and held the tentacles in a fixed position. If the ROV maintained its distance, the juvenile continued alternating between swimming tail down and hovering tail down as previously described. However, when the ROV continued to get closer and eventually touched the juvenile *C. calyx*, it retracted its tentacles, put all of its arms together, and jetted backwards while expelling a pseudomorph of ink. When touched again, the juvenile repeated the jetting and ink release, but changed the directions of the jetting and inking multiple times. Finally, the distressed juvenile *C. calyx* autotomized its entire tail and jetted/swam away forwards or backwards while dark.

A subadult *C. calyx*, characterized by its lack of a tail, was typically pale when encountered and in, under, or near a cloud of ink in its oblique fishing posture. In this posture, its tentacles were extended and hanging beneath the body with its arm tips curled. When alarmed, the subadult retracted its tentacles and darkened the regions of its body surrounding the digestive gland, eyes, and photophores. Further disturbance caused it to slowly swim down in a forward or backward orientation with its arms together. If the ROV maintained its distance, the subadult eventually stopped swimming down and oriented back into a pseudo-fishing posture: hovering horizontally without re-deploying its tentacles and maintaining its darkened regions. The subadult *C. calyx* repeated these behaviors until the ROV got even closer; at this point, it darkened all chromatophores and hovered, remaining still. When touched, the subadult immediately started alternating between pale and dark and rapidly spread its arms and extended its tentacles while simultaneously illuminating the photophores in these regions.

ORIENTATION STUDY OF *C. calyx* AND *N. bijuga*

Nanomia bijuga examined in the morning (06:30 to 09:30) were mostly be oriented siphosome up above 300 m in depth and siphosome down below 300 m as indicated in *Figure 1*. Similarly, *N. bijuga* observed from 13:00 to 18:00 were found to mainly orient siphosome down below 300 m in depth, but mostly oriented indeterminately above 300 m as displayed in *Figure 1*. Interestingly, *C. calyx* juveniles were also found to mostly orient tail down below 300 m in depth whereas subadult *C. calyx* were equally distributed across the three orientations below 300 m as indicated in *Figure 1*.

DISCUSSION

C. calyx exhibited a staggering array of postural, chromatic, bioluminescent, and locomotor behaviors comparable to or exceeding ethograms established for shallow water cephalopods. Seventy-three behavioral components and 5 behavioral sequences are now described for *C. calyx*. This wide array of behaviors adds to the evidence contradicting previous predictions that the visual components of deep sea cephalopod behavior would be limited to transparency, silvering, red coloration, counter-illumination, and bioluminescence (Bush, et al., 2009). Instead, it is clear that *C. calyx* performs many complex behavioral sequences potentially aiding in interspecifc and intraspecific communication (Bush, et al., 2009). Moreover, it is likely that the behaviors we describe only constitute what is likely a small portion of the total number of behaviors performed by *C. calyx*, as the behaviors we describe can primarily be considered defensive responses to the presence of the ROV (Hunt, 1996). While ROV use has vastly increased our knowledge of behavior in the deep sea, and will continue to do so, it also has the drawback of being a rather intrusive method of studying behavior. ROVs are large, noisy, and bright and, not surprisingly, illicit defensive responses in animals with the sensory mechanisms capable of detecting the ROV's presence (Hunt, 1996). Nevertheless, the ROV footage enabled us to identify and describe many remarkable behavioral adaptations *C. calyx* possesses that aid in evading the visually-cued predators of the mesopelagic zone.

The propensity of *C. calyx* to remain still, or only move very slowly when threatened, is one of the first behavioral traits one might notice upon an encounter. *Chiroteuthis calyx* has been previously noted by Bush and Robison, 2007 to be slow to react to the ROV's presence and is frequently anthropomorphized by others as being "lazy."We not only found *C. calyx*, during ROV observations, to be slow to react to the presence of the vehicle, but to also not be inclined to rapidly swim or jet away until physically touched by the ROV. This propensity to move as little as possible when threatened is likely not only an energy-conserving adaptation to life in the mesopelagic zone where food and oxygen are scarce, but also an attempt to remain concealed from visually-oriented predators in a habitat where bioluminescence is so prevalent (Herring, 2002; Robison, 2004; Dr. Sean Lema, pers. comm.).

Additional locomotor behaviors also seem to confer success at predator avoidance. We observed *C. calyx* performing 5 different ink release behaviors, with the pseudomorph ink release being the most common in juveniles (n=22) and the cloud ink release (n=13) being the most common in subadults. It appears as if inking juveniles produce the pseudomorph ink release as an alternate target for visually-cued predators. The clouds produced by subadults may be used as a structure to hide behind (Bush and Robison, 2007). Similar to the squid *G. onyx*, which is thought to mimic elongate siphonophores with its rope ink release (Bush and Robison, 2007), it appears as if juvenile *C. calyx* also mimic elongate siphonophores with their pseudomorph series ink release (n=3) and rope ink release (n=1) that we describe. On the few occasions that we witnessed juvenile *C. calyx* performing these behaviors, we always additionally noticed elongate siphonophores in the near vicinity. It has also been speculated by Bush and

Robison, 2007 that the single pseudomorph ink release perfomed by *C. calyx* could additionally be an attempt to mimic the siphonophore, *N. bijuga*. The pseudomorph ink release in juveniles is often accompanied by hovering or jetting. When *C. calyx* hovers to remain still, the wavering band of ink can remain next to the animal (n=5), effectively doubling its length (Vecchione, et al., 1992; Bush and Robison, 2007).

The bioluminescent behaviors that we observed in *C. calyx* seemed primarily reserved for predator avoidance and camouflage, although they undoubtedly use the photophores on their 4th arms, tentacle stalks, and tentacle clubs for prey attraction (Hunt, 1996). It was impossible to view the actual light production of this animal under the bright lights of the ROV. Instead, it was assumed that if the photophores on the animal were exposed, they were producing light. Across both age groups of *C. calyx*, the ocular and visceral photophores were always oriented down and exposed no matter what orientation the animal was in. This indicates that these photophores serve in the function of counter-illumination and thus camouflage. Aside from prey attraction, the photophores on the 4th arms and tentacles seemed to have the function of predator distraction by functioning as an alternate target for visually-cued predators, similar to the bioluminescent arm tips of *O. deletron* (Bush, et al., 2009). The exposure of these photophores occasionally coincided with the rapid spread of the 4th arms or extension of the tentacles when the animal was touched by the ROV.

Chromatic body patterning in deep sea cephalopods has been hypothesized to function in predator avoidance and prey acquisition (Bush, et al., 2009). Based on the brown orange to dark red color of the chromatophores in *C. calyx*, it appears that they function in concealing the part of the body that they cover, as these wavelengths of light are not present in the mesopelagic zone (Robison, 1999; Herring, 2002). Accordingly, there are dense concentrations of chromatophores located on *C. calyx* in all areas possessing photophores and these areas are often the first to darken when the animal feels threatened by the presence of the ROV (Vecchione, et al., 1992; Hunt, 1996). Chromatophores could also be used to produce disruptive coloration, helping to break up the characteristic body shape of *C. calyx* and thus make them less recognizable to visually-cued predators while keeping them hidden from their prey while foraging (Bush, et al., 2009). Finally, chromatophore use is most likely important in prey attraction in *C.*

calyx. Hunt, 1996 and Bush, et al., 2009 noted that chromatophores covering bioluminescent organs on the tentacles and arms of deep sea squid can be rapidly expanded and contracted, effectively flashing the photophores. This could be used as a form of prey attraction, or possibly communication (Hunt, 1996; Bush, et al., 2009).

Juvenile *C. calyx* are often found in these vast swarms of *N. bijuga* and occur in the same depth range that the siphonophores do. It is also likely that juveniles feed on the same animals that *N. bijuga* do. Not only is the tail of juvenile *C. calyx* very similar in appearance to the siphosome of *N. bijuga* (Vecchione, et al., 1992; Hunt, 1996; Seibel, et al., 2004; Bush and Robison, 2007; Roper and Young, 2013), but juvenile *C. calyx* also appear to orient their tail in the same manner as the surrounding *N. bijuga* orient their siphosome. The results of our orientation study indicate corresponding trends in *N. bijuga* orientation and juvenile *C. calyx* orientation. Below 300 m, it is more common to find *N. bijuga* oriented with their siphosome down. There was no pattern to how subadult *C. calyx* oriented in the water column. In contrast, juveniles mostly oriented tail-down in the water column, below 300 m. Body orientation in *C. calyx* juveniles could confer mimicry of this highly abundant and active siphonophore. Because siphonophores possess powerful nematocysts and lack sufficient nutrient density to warrant attack from the predators of mesopelagic squid (Vecchione, et al., 1992; Robison, 1999), *C. calyx* juveniles would most likely experience reduced predation as a result of behaviorally and morphologically mimicking *N. bijuga*.

Tail autotomy by juvenile *C. calyx* constitutes a last-resort defense (Bush, 2012), offering not only a target for visually-cued predators, but also a possible target for acoustically-oriented predators, such as cetaceans (Clarke, 1996; Dr. Bruce Robison, pers. comm.). It was clear that the tail could either be unintentionally broken somewhere along its length and/or removed entirely via contact with the ROV, or could be intentionally released by a particularly disturbed individual (pers. observ.). Upon removal, we observed the tail to remain stationary in the water column, thus exhibiting neutral buoyancy. Autotomized tails were also observed to retain the chromatic pattern that it had when released or removed from the individual. Tail autotomy appears to be followed by a change in locomotor behavior. We observed that individuals without tails were more likely to swim forwards (arms-first) than individuals with tails. Bartol, et al., 2001 found that for the squid, *Lolliguncula brevis*, arms-first swimming is preferable at lower speeds and tail-first swimming is preferable at high speeds. While tail-first swimming allows for more control at faster speeds, arms-first swimming allows the squid to better observe its forward surroundings since the eyes are located towards the anterior end of the animal and the arms can be manipulated to provide a better view (Bartol, et al., 2001). Regardless of orientation, individuals of both age classes we more often observed to swim toward greater depths in response to the presence of the ROV than towards shallower depths (Hunt, 1996).

When *C. calyx* juveniles lose their conspicuous tail, this component of their morphological transformation is, not surprisingly, accompanied by a behavioral change. Juvenile *C. calyx* and subadult *C. calyx* not only have a significantly different suite and incidence of postural and bioluminescent behavioral components, but also appear to differ in their chromatic and locomotor behaviors. While we noted many behavioral sequences, only 5 were common enough to warrant description in our ethogram. All will benefit by further video-analysis, but we noted some interesting trends: Juvenile *C. calyx* are more frequently seen participating in behavioral sequences involving a tail-down orientation than subadult *C. calyx*. These include three out of the five behavioral sequences we described. We found that juveniles also have an entirely different response to being touched by the ROV. Recent research also shows that the diet of *C. calyx* drastically changes with age (Anela Choy, pers. comm.). In fact the delta n values suggest that subadult *C. calyx* shift their diet up an entire trophic level from the juvenile life stage. This finding suggests that there are, in fact, many more behaviors yet to be seen in *C. calyx*.

CONCLUSIONS/RECOMMENDATIONS

Chiroteuthis calyx is capable of performing numerous postural, chromatic, bioluminescent, and locomotor behaviors. The morphological transformation from the juvenile to subadult life stage in *C. calyx* is accompanied by a behavioral change. Behavioral sequences that are more common in the juvenile life stage of *C. calyx* enable it to closely resemble *N. bijuga*. Juvenile *C. calyx* tend to orient structures akin to those present on *N. bijuga* in the same manner that this siphonophore does, whereas subadults, not possessing these structures, do not orient in a predictable fashion.

Re-entering the data in a fashion that treats every individual examined as its own data point rather than lumping all the animals we observed together will enable us to perform more accurate statistical tests. Further video analysis, particularly of subadults, will aid in identifying behavioral sequences and also add power to our statistical comparisons of the behavioral differences between juveniles and subadults. Additional observations of *C. calyx* also could be added to the orientation study by further video observation. Images of *C. calyx* should be added to this paper and videos of *C. calyx* behavior that have already been made could be posted online for reader reference. Edits also should be made to sections of this paper, particularly the discussion and abstract.

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