On the Biology, Behavior, and Conservation of the Chambered Nautilus, Nautilus sp.

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ON THE BIOLOGY, BEHAVIOR, AND CONSERVATION OF THE
CHAMBERED NAUTILUS, NAUTILUS SP.

By

Gregory Jeff Barord

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2015
This manuscript has been read and accepted for the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

ON THE BIOLOGY, BEHAVIOR, AND CONSERVATION OF THE CHAMBERED NAUTILUS, *NAUTILUS* SP.

By

Gregory Jeff Barord

Advisor: Dr. Jennifer Basil

Chambered nautiluses are unique molluscs that differ from their closest relatives, octopus, squid, and cuttlefish, in many ways. Most obvious, nautiluses possess the ancestral trait of an external shell; a shell that has protected them for hundreds of millions of year but is dooming them today. Although nautiloids have survived all five mass extinction events, the lineage is under siege in the current ‘sixth mass extinction’. Unregulated, unmanaged, and ongoing nautilus fisheries, particularly in the Philippines and Indonesia, have been depleting populations in a matter of years, some to local extinction, to supply a worldwide demand for the ornamental shell. Although nautiluses are heavily fished and common in the shell trade, there is a considerable lack of information on their biology, ecology, and behaviors. Thus, at this point, we do not have enough basic information about nautiluses to propose management plans and conservation practices. Here, we investigate three aspects of nautilus life history as it relates to conservation by combining laboratory and field studies: navigational tactics, feeding behaviors, and population demography.

Nautiluses learn and remember visual cues to find a goal using a beacon, or constellation of cues around the goal. However, the contribution of kinesthetic, or route memory, as they
navigate to the goal, is unknown. Here, we tested the nautiluses’ ability to navigate a maze by shifting or removing a visual beacon cue used to identify the goal. We found that after learning that a beacon cued a goal in a spatial maze, nautiluses switched to route memory to find the goal when the beacon was removed. However, this switch was difficult for them. Nautiluses tested with a shifted beacon, 45° relative to the goal, ignored their route memory to orient toward the beacon instead. Only when the beacon was shifted 90° from the learned location, or was removed entirely, did the animals seem to switch to route memory. Thus, it appears that during learning, the beacon overshadows the acquisition of route memory. However, as animals were successful in finding the goal when the beacon was removed, overshadowing was not complete – nautiluses were able to access route memory when the beacon was removed entirely. Thus, nautiluses exhibit behaviors that indicate they are adapted for an environment with cues that may shift or become unreliable.

Most cephalopods are active predators that rely on a suite of different behaviors to capture live prey. Nautiluses have been characterized as predators, scavengers, and opportunistic scavengers, among other terms. However, no direct evidence has been available to confirm these claims. Here, we used field and laboratory observations to describe what type of prey nautiluses prefer (dead or live) and how they locate and capture prey items. In the field, baited remote underwater video systems (BRUVS) were deployed at four different sites in the South Pacific to depths of 300-400m to record feeding behavior of wild *Nautilus*. In the laboratory, a mock setup of nautilus habitat was used to test and record the nautiluses’ ability to locate and capture dead, and sometimes buried, shrimp. In both settings, the nautiluses exhibited the same foraging behaviors. Remote tracking of the food source was characterized by the *cone of search* behavior with tentacles extended outward and laterally. Field observations suggested that nautiluses may
dig for prey items and laboratory experiments confirmed this ability. Nautiluses were able to
locate prey from a distance and then excavate buried prey items. An unexpected result here was
that nautiluses showed no foraging or predatory behaviors toward live prey items in the field
which suggests that nautiluses may only forage on decaying prey. The foraging and digging
behaviors appear to be fixed action patterns in *Nautilus*, exhibited in the presence of odor stimuli
whether the nautilus consumes the food item or not. This would be an ideal adaptation for an
opportunistic forager finding food in a dark environment with limited prey items. However, this
adaptation has the secondary effect of leaving nautiluses highly vulnerable to being caught in
traps baited with dead prey items. In addition, their digging behavior makes them susceptible to
accumulating toxins in the sediment that may collect on the ocean floor as a result of increasing
coastal development and runoff.

The extant species of *Nautilus* and *Allonautilus* (Cephalopoda) inhabit fore-reef slope
environments across a large geographic area of the tropical western Pacific and eastern Indian
Oceans. While many aspects of their biology and behavior are now well-documented,
uncertainties concerning their current populations and ecological role in the deeper, fore-reef
slope environments remain. Given the historical to present-day presence of nautilus fisheries at
various locales across the Pacific and Indian Oceans, a comparative assessment of the current
state of nautilus populations is critical to determine whether conservation measures are
warranted. We used baited remote underwater video systems (BRUVS) to make quantitative
photographic records as a means of estimating population abundance of *Nautilus* sp, at sites in
the Philippines Islands, American Samoa, Fiji and along an approximately 125 km transect on
the fore reef slope of the Great Barrier Reef from east of Cairns to east of Lizard Island,
Australia. Each site was selected based on its geography, historical nautilus abundance, and the
presence (Philippines) or absence (other sites) of *Nautilus* fisheries. We found significantly fewer nautiluses with this method than expected in the Philippine Islands site. While there may be multiple reasons for this difference, the most parsimonious is that the Philippines Islands population has been reduced due to fishing. Specifically, historical trap records from the same site demonstrate there have been far more nautiluses at this site in the past.

Effective conservation plans benefit both the species of interest as well as the community. We identify visual and kinesthetic cues and tactics that are important to nautiluses returning to locations in their habitat (e.g., hiding spots, good foraging), and support the hypothesis that nautiluses are strict scavengers, sometimes reliant on digging in the substrate to find food they have found using olfactory cues. We also report on the health of populations in both fished and unfished sites in the Indo Pacific. There is still work to perform, such as identifying preferred habitat type, preferred species of prey, and calculating abundance levels at different areas and at different times. However, without protection, fisheries will continue to deplete nautiluses to extinction, one population at a time, as the fishermen move to new sites when one site is no longer profitable.
Acknowledgments

I thank God for His many blessings and challenges.

My parents, Mom and Dad as I know them, Laurel and Paul to everyone else, supported me every step of the way in every way possible. I can only imagine what they were really thinking when, as a five year old, I told them I wanted to study squid and get a PhD.

My little bro, Ryan, was always there to make fun of me wanting to study squid and the ocean. But I had the last laugh because he will never know I always let him win in basketball, until now.

My best friend/girlfriend/research partner, Karen, has been through so many ups and downs throughout it all. It is a special thing to be able to share the work that you love with the person that you love. Although she can’t love nautiluses as much as me, I’m glad she puts up with me.

To all my other family and friends, they’ve all been there for interesting points along the way, they’ve all give support and well wishes in their own way. From coming up with kind nicknames because of my love for squid and nautiluses (thanks Ross and Danny) to being there when I gave my first presentation (Thanks Aunt Gretchen and Uncle Ed).

To my adviser, Dr. Jennifer Basil, for taking on a kid who wanted to study nautiluses but knew nothing about behavior. The opportunities I’ve had as a result of Jenny have been life changing in so many ways. She’s gone out of her way to support all of my passions and help me along the way so that as I ready myself to finish my dissertation, I have no regrets. Thanks!

My advisory committee (in no particular order) has been both terrifyingly frightening (in a great way) and an incredible resource and support system. The frightening part is simply wanting to produce the best work possible for them. There are only so many folks working on nautiluses and I managed to squeeze three on my committee: Dr. Peter Ward, Dr. John Chamberlain, and Dr. Heike Neumeister. Peter has become a great colleague and friend who still puts up with me on research trips throughout the South Pacific. John has always offered advice and support at Brooklyn College and some interesting projects and ideas to look into. Heike is so passionate and quantitative about nautiluses, particularly saving them, that it has rubbed off on me a lot. Dr. Phillip Lee, my undergraduate adviser, helped me through the ways of keeping a cephalopod alive in an aquarium, something not so easy. Beyond that, he’s always given honest support and help along the way. Dr. Eugenia Naro-Maciel helped to guide me through the real definition and meaning of conservation biology with different perspectives. Dr. David Lahti made me think about things from an even different perspective, even if we didn’t want to. Dr. Roland Anderson would have certainly been on my committee if he were still with us. I miss our talks about many things, especially ‘enrichment’ and the potential of putting a sea lion in a GPO tank. I miss him.

I dedicate this work and my career to my Uncle James and Uncle Jeff. Uncle James was always talking to me about marine biology and helping me out when I had questions about my own tanks. Even when he was battling cancer and going through chemotherapy, he always answered my questions about this fish or that fish. I was never able to meet Uncle Jeff but from what my Mom tells me, he would have helped me celebrate this awesome day the right way!
Table of Contents

Abstract ......................................................................................................................... iv
Acknowledgments ....................................................................................................... vii
List of Tables ............................................................................................................... ix
List of Figures ............................................................................................................. x
Chapter 1. Introduction to Nautiluses
  1.1 Nautilid Evolutionary History ................................................................. 1
  1.2 Biology of Nautiluses ............................................................................. 2
  1.3 Nautilus Behavior ................................................................................... 4
  1.4 Conservation Status of Nautiluses ......................................................... 5
  1.5 Summary .................................................................................................... 6
Chapter 2. Nautilus Homing: Integrating Different Homing Cues
  2.1 Introduction ............................................................................................... 7
  2.2 Materials and Methods .......................................................................... 10
  2.3 Results ....................................................................................................... 15
  2.4 Discussion .................................................................................................. 23
Chapter 3. Foraging and Scavenging Behavior in Nautilus: A Field and Laboratory Study
  3.1 Introduction ............................................................................................... 27
  3.2 Materials and Methods .......................................................................... 30
  3.3 Results ....................................................................................................... 37
  3.4 Discussion .................................................................................................. 47
Chapter 4. Comparative Population Assessments of Nautilus sp. in the Philippines, Australia, Fiji, and American Samoa Using Baited Remote Underwater Video Systems
  4.1 Introduction ............................................................................................... 56
  4.2 Materials and Methods .......................................................................... 59
  4.3 Results and Discussion .......................................................................... 62
  4.4 Conclusion .................................................................................................. 65
Chapter 5. General Discussion ................................................................................. 67
Chapter 6. Future Considerations ............................................................................. 72
Literature Cited ......................................................................................................... 73
List of Tables

Table 2.1 Mean trial latency for nautiluses in training trials of three different conditions.  15
Table 2.2 Means of total distance traveled during training trials in each conditions……  21
Table 3.1 Overall data of number of video hours recorded and total number of nautiluses
recorded at each of the survey sites……………………………………………………………  31
Table 4.1 Population abundance data at each of the sampling sites…………………..  64
List of Figures

Figure 1.1 General anatomy of the chambered nautilus, *Nautilus pompilius*, as represented here from a specimen collected in Panglao, Philippines……………………………………... 2

Figure 1.2 Radiograph of *Nautilus pompilius* showing the internal chambers that function in buoyancy control for nautiluses………………………………………………………... 3

Figure 2.1 Closed recirculating marine system used to house *Nautilus pompilius* at Brooklyn College……………………………………………………………………………………………………... 10

Figure 2.2 Representation of the different experimental trials with the varied location of the beacon during the probe trials…………………………………………………………………... 12

Figure 2.3 Representation of different arena maze setups for training and test trials…. 13

Figure 2.4 Latency of *Nautilus pompilius* to complete the task during the five training trials in the three different conditions with a beacon present during training………………... 16

Figure 2.5 Average time difference between trial five and the probe trial among the three beacon present training groups………………………………………………………………... 17

Figure 2.6 Latency to find goal for successful outcomes during the three different probe conditions………………………………………………………………………………………………... 18

Figure 2.7 Percentage of successful outcomes of *Nautilus pompilius* during the three probe conditions when trained with a beacon thought we were eliminating this………………. 19

Figure 2.8 Navigational paths of nautiluses in training and probe conditions……………… 20

Figure 2.9 Nautilus navigational path in in trial one versus trial five…………………... 20

Figure 2.10 Total distance traveled during training trials…………………………………… 22

Figure 2.11 Average change in distance traveled between trial 5 and probe trials……... 22

Figure 2.12 Average total distance traveled during probe trials………………………… 23
Figure 3.1 Diagram of the baited remote underwater video systems frame………………... 32
Figure 3.2 Diagram of the closed recirculating marine system used to house nautiluses at Brooklyn College………………………………………………………………………………. 34
Figure 3.3 Lateral view of experimental tank …………………………………….. 35
Figure 3.4 Top view of experimental tank displaying the position of the cameras and the four different quadrants the shrimp was randomly placed in…………………………………… 36
Figure 3.5 Snap shot from baited remote underwater video systems showing Nautilus pompilius displaying typical cone of search behavior towards the bait source used in Australia… 38
Figure 3.6 Snap shot from baited remote underwater video system showing a different Nautilus pompilius displaying cone of search behaviors in Australia………………………………….. 39
Figure 3.7 Single Nautilus pompilius in American Samoa displaying cone of search behavior from at least 5 m from the bait source as it moves closer……………………………………. 40
Figure 3.8 Nautilus pompilius in Philippines feeding on the bait while live prey items, specifically shrimp, crawl on the nautilus’ shell and hood…………………………………… 41
Figure 3.9 Nautilus pompilius in American Samoa jetting around the bait with multiple shrimp crawling on its hood and shell. Nautilus showing no predatory behaviors to shrimp….. 41
Figure 3.10 Four distinct foraging and scavenging stages (a-d) of Nautilus pompilius when searching for shrimp placed on substrate during training trials…………………………. 43
Figure 3.11 Latency of Nautilus pompilius to locate the shrimp during training ……… 44
Figure 3.12 Four distinct foraging and scavenging stages (a-d) of Nautilus pompilius when searching for shrimp buried under the substrate during testing………………………… 45
Figure 3.13 Latency of Nautilus pompilius to locate the shrimp during testing trials….. 47
Figure 3.14 Illustration of a possible mode of search patterns in *Nautilus pompilius* after detection of an olfactory cue in the wild………………………………………………………… 50
Figure 3.15 Illustrated model of the general stages of nautilus scavenging…………….. 53
Figure 4.1 Snap shots of *Nautilus* sp. taken during underwater video observations…. 63
Chapter 1. Introduction to Nautiluses

1.1 Nautilid Evolutionary History

The first cephalopods appeared in the middle to late Cambrian (Teichert, 1988; Dzik, 1981), with the nautilid lineage arising 500 million years ago (Kroger et al., 2011). Extant nautiluses are grouped in two genera, *Allonautilus* and *Nautilus* (Ward and Saunders, 1997). *Allonautilus scrobiculatus* is the only species within the genus, whereas *Nautilus* comprises several different populations. Based upon morphology, a total of five to six species have been described in the literature (Woodruff et al., 1983; Saunders et al., 1987; Bonnaud et al., 2002, 2004). However, recent phylogenetic analysis suggests that *Allonautilus scrobiculatus* and *Nautilus macromphalus* are the only two phylogenetically distinct species while the common *Nautilus pompilius* is a paraphyletic species with genetic differentiation correlated to geographic distribution (Bonacum et al., 2011). The lack of genetic differentiation in *Nautilus* may be a result of nautiluses currently undergoing an adaptive radiation throughout their habitat in the Indo-Pacific (Bonacum et al., 2011). *Nautilus pompilius*, however, is still used to refer to these animals in public aquaria and research institutions and will be used here.

Nautiluses are found along deep coral reef slopes of the Indo-Pacific Ocean and their habitat is regulated by warm ocean temperatures near the surface (Martin et al., 1978; Carlson, 1979) and depth implosion limits below (Saunders and Wehman, 1977; Ward and Martin, 1980; Kanie et al., 1980, 1981; Kanie and Hattori, 1983; Chamberlain and Chamberlain, 1985). Nautiluses may also remain close to the ocean floor, within 1 m, to reduce predation (Saunders and Ward, 1987). These habitat restrictions significantly reduce gene flow between populations and contribute to the current adaptive radiation of the separate populations. In addition, these barriers to migration also affect their ability to respond to over fishing.
1.2 Biology of Nautiluses

1.2.1 Anatomy

Nautiluses are members of class Cephalopoda and are the only living representatives of this class that still possesses an external shell (Figure 1.1), which differentiates them from the coleoid cephalopods (octopuses, squid, and cuttlefish). The external shell of nautiluses functions in protection and buoyancy control and is composed of many internal chambers (Figure 1.2) that contain gas and cameral fluid (Denton and Gilpin-Brown, 1966; Ward et al., 1977). Nautiluses possess up to 90 tentacles without suckers. Instead, each tentacle has numerous grooves that
secrete a sticky mucopolysaccharide (Kier, 1987; von Byern, 2012) that allows them to grasp prey or mates. The eye of nautiluses is referred to as a “pinhole camera” and lacks a lens that is characteristic of the coleoid cephalopods (Hurley et al., 1978; Muntz and Raj, 1984). Thus, without a lens, among other differences, the vision of nautiluses is not thought to be as acute as octopus, squid, or cuttlefish (Muntz, 1986; Muntz and Wentworth, 1987). Little is known about their use of vision in the wild and we address that question here.

Figure 1.2 Radiograph image of *Nautilus pompilius* revealing the internal chambers and siphuncle running through the middle of the chambers that function in buoyancy.
1.2.2 Reproduction

Nautilus are *k-selected species* and exhibit long life spans (of at least 10-15 years; Landman et al., 1988; Dunstan et al., 2011), long developmental times (1 year; Okubo et al., 1995; Uchiyama and Tanabe, 1996), delayed maturity (Saunders, 1983; Saunders, 1984; Landman et al., 1994), and low fecundity (up to 10 eggs; Okubo et al., 1995; Uchiyama and Tanabe, 1996). While these reproductive strategies may be advantageous to living in the deep-sea ecosystem, they compound the effect of removing individuals from a population with no ability to quickly replace members reproductively, or by migration in from elsewhere.

1.3 Nautilus Behavior

Nautilus behavior has not been studied thoroughly, potentially because of their relatively smaller and less complex brain than coleoids (Young, 1965; Maddock and Young, 1987; Shigeno et al., 2008; Grasso and Basil, 2009) in addition to their deep-water habitat and solitary lifestyle. However, the nautilid brain is still a relatively large brain when compared to other molluscs, and as a long-lived group, complex behavior involving learning and memory is likely. Recent evidence shows that nautiluses do in fact display complex behaviors, including short and long-term memory (Crook and Basil 2008), long-term spatial memory (Crook et al., 2009), three-dimensional spatial memory (Crook et al., 2009) and complex, dynamic spatial tactics when homing in an uncertain environment (Crook and Basil, 2013). In many ways, their behaviors were surprisingly similar to coleoids (Boal et al., 2000; Crook and Basil, 2008; Crook et al., 2009; Crook and Basil 2013). Much still remains to learn about their behaviors both in the field and under controlled laboratory conditions.
An example of a difficult behavior to quantify and observe in the field is natural foraging behavior. Most coleoids, except the vampire squid (Hoving and Robinson, 2012), are active predators that hunt live prey (see review in Hanlon and Messenger, 2010). The foraging patterns of nautiluses are less clear. There are several suggestions that nautiluses are scavengers (Bidder, 1962; Kier, 1987; Saunders, 1987; Basil et al, 2000; Ruth et al., 2002; Basil et al., 2005) but nothing is known about whether they also hunt and consume live prey. Identifying behaviors nautiluses use to scavenge, or if they scavenge at all, in semi-naturalistic laboratory study allows us close observation of behaviors that are difficult to observe in the field, while providing comparison to field behaviors, when possible, to document similarities. The field is therefore at a stage where a unique combination of novel tools developed for the field and controlled laboratory study allow us to answer long-standing questions about nautilid biology and we combine these approaches here.

1.4 Conservation Status of Nautiluses

The current status of most populations of nautiluses is unknown. Nautiluses have been fished for nearly 50 years with little to no regulation, not for their meat as a food source, but for their ornamental shell which is sold world-wide (DeAngelis, 2012). Although anecdotal reports and observations suggested a decline in nautilus populations since the creation of fisheries, these reports have not been quantified scientifically. Thus, management and conservation initiatives have been stymied. The first scientific study of nautilus populations, comparing a non-fished site in Australia to a fished site in the Philippines (Dunstan et al., 2010) suggested a negative impact of nautilus fisheries. Dunstan et al. (2011) developed a new method to assess the status of nautilus populations using underwater cameras that record the number of nautiluses attracted to a
bait source. Dunstan et al (2011) found that a non-fished population in Osprey Reef, Australia remained stable over a 10-year sampling period and was naturally small. These findings suggest that even *healthy* populations are vulnerable to over exploitation and provided the first baseline data of current nautilus populations.

1.5 Summary

The last remaining living members of the nautilid lineage are in a precarious situation as fisheries continue (Dunstan et al., 2010) and there are no international agreements to protect or regulate the trade (DeAngelis, 2012). In addition to questions of nautilus population size, there are still many unknowns about their general biology and behavior which would help inform conservation efforts. My goal here is examine three aspects of their biology (navigation, foraging, and population demography) to better inform future management plans to ensure that nautiluses are not fished to extinction. A perhaps more accurate, and troubling, description given their unique life history and ecology is “mined to extinction” (Dunstan, personal communication).
Chapter 2. Nautilus Homing: Integrating Different Homing Cues

2.1 Introduction

Nautiluses are a unique group of marine organisms whose lineage can be traced back nearly 500 million years (Kroger et al., 2011). Often referred to as ‘living fossils’ because of their morphological resemblance to their fossilized ancestors, nautiluses are the only living cephalopods that still possess an external shell. There are two currently recognized genera of nautiluses, *Nautilus* and *Allonautilus* (Ward and Saunders, 1997), although the exact number of species is still not clear.

The learning and memory capabilities of coleoid cephalopods (Octopus, squid, and cuttlefish) are well documented (Boycott and Young, 1950; Messenger, 1973; Messenger, 1977; Agin et al., 1998; Hochner et al., 2003; Darmallaiq et al., 2004; Boal et al., 2005; Darmallaiq et al., 2006; Alves et al., 2007; Alves et al., 2008; Darmallaiq et al., 2008; Hanlon and Messenger 2010). However, learning and memory in nautiluses has only recently been investigated and described (Crook, 2008; Crook et al., 2009; Basil at al., 2012; Crook and Basil 2013; Basil and Crook, 2015). Among the many morphological and physiological differences between nautiluses and coleoids, one major difference is the complexity of the central nervous system. Coleoids possess distinct vertical and suprafrontal lobes that are associated with learning and memory (Young, 1960; Young, 1961; Fiorito and Chickery, 1995; Dickel et al., 1997; Dickel et al., 1998; Dickel et al., 2001; Langella, 2005; Hochner et al., 2006; Shomrat et al., 2008). However, the nautilus brain is simpler, containing fewer lobes than the coleoid brain (Young, 1965; Maddock and Young, 1985; Maddock and Young, 1987; Grasso and Basil, 2009) and also represents the ancestral neural condition of cephalopods (Young, 1991; Shigeno et al., 2008; Sasaki et al., 2010; Shigeno et al., 2010). While nautiluses do not possess the specialized vertical lobe...
dedicated to learning and memory found in coleoids (Young, 1965; Grasso and Basil), another area of their brain may underlie their learning and memory capabilities (Crook and Basil, 2008). Having a living representative of the ancestral condition to compare with coleoids is a unique opportunity to understand evolutionary and ecological contributions to brain complexity (Grasso and Basil, 2009; Basil et al., 2012). However, much remains unknown about fundamental aspects of the behavior of nautiluses and we address their ability to process visual information to navigate and to home here.

In their natural habitat, nautiluses perform daily vertical and horizontal migrations (Carlson et al., 1984; Ward et al., 1984; Dunstan et al., 2011). Although these migration patterns are variable between geographic locations, possibly related to the local habitat, these daily patterns are likely supported by the ability to home and navigate to known locations, such as foraging sites, hiding spots, and areas to lay eggs in safety. In the wild it is difficult to examine how nautiluses navigate their environment because of the extreme depths they inhabit. Thus laboratory study enhances our ability to understand the tactics they use to navigate in the wild.

Nautiluses readily learn to navigate in two and three dimensional spatial mazes to find a goal (Crook et al., 2009; Crook and Basil 2013). Nautiluses have been trained to locate the exit hole of a shallow-water maze, to deeper water, cued by a black and white striped beacon. After learning to home successfully and quickly to the beacon, the animals were tested from two hours to three weeks after their last time in the maze to determine how long the nautiluses remembered the solution to the task. Nautiluses remembered the solution to the maze (the beacon) for two weeks and possibly up to 21 days (Crook et al, 2009). This memory retention is on par with that found in similar studies with coleoid cephalopods (Boal et al., 2000; Basil et al., 2012) despite
their neural differences. Nautiluses also learn and remember the 3 dimensional contours of an artificial coral reef (Crook et al., 2009) using a combination of visual and tactile features.

Nautiluses were then tested with multiple landmarks distal to the goal to identify the suite of navigational tactics nautiluses use to find a goal (Crook and Basil, 2013), including route memory without visual cues (i.e., body position in space, direction, etc.), beacon-based homing, or geometric relationships between multiple landmarks and their relationship to the goal. Nautiluses were able to dynamically switch homing tactics, using a beacon, the geometric array of maze cues, and even extra-maze cues to find the goal. The role of route memory, however, was less clear. When the beacon was removed and no other cues remained, nautiluses had difficulty finding the goal. However, in later studies with more training (Crook and Basil, 2013), nautiluses eventually could compensate with route memory when the beacon was removed -- suggesting the two cues share a memory stream, and may compete with one another for memory space (Rescorla and Wagner, 1972).

In the wild, nautiluses must cope with a variety of environmental cues to determine where they are and where they need to go – some variable and some reliable. Little is known about how this is accomplished in the field, so these questions are perfectly positioned to be answered in the laboratory. For instance, how do nautiluses learn and remember multiple cues and use them interchangeably? Are the cues learned simultaneously in the same memory stream and accessed flexibly depending on what is available later on? Or are the different sources of information coded separately, perhaps stored in different areas of the brain? The following experiment examines how multiple cues are learned and then remembered using the simple beacon homing maze from Crook et al., (2009). Here we test for their ability to 1) detect that the beacon has moved, and when it is rendered unreliable, and 2) if and/or how they are integrating
simultaneously learned route-memory information to find the beacon. Specifically, does learning the beacon make accessing route memory more difficult later on (shared memory space) or not (separate memory streams)?

2.2 Materials and Methods

2.2.1 Nautilus Husbandry

The successful husbandry of chambered nautiluses requires effective system design, specialized equipment, and consistent monitoring of health. The high metabolism of all cephalopods means that water quality can quickly deteriorate in a closed system (O’Dor et al., 1993). In addition, the microvillus epidermis of cephalopods results in a high surface area potential to absorb toxins faster and at lower levels. Thus, the holding system must be designed to effectively reduce initial nitrogenous waste (ammonia – NH$_3$) into lesser toxic by-products (nitrite – NO$_2$ and nitrate – NO$_3$). This includes a combination of biological, mechanical, and chemical filtration (Carlson, 1987; Spinosa, 1987; Lee et al., 1998; Barord and Basil, 2014). Nautiluses also require chilled seawater as they will die quickly in temperatures approaching 27°C (Carlson, 1987) so a refrigerated chilling unit is necessary to maintain temperature (Carlson, 1987; Barord and Basil, 2014). However, the most effective husbandry practice for nautiluses (and all cephalopods) may simply be acute observations (Oestmann, 1997).

The nautiluses used for the spatial study were wild caught *Nautilus pompilius* from the Philippines and supplied by SeaDwelling Creatures Inc. All of the animals were housed in a 2625 l system (Figure 2.1, color coded). The system included three cylindrical holding tanks (1.5 m tall, 1 m diameter), a 187.5 l sump holding biological filtration, a mechanical pump (black), a chilling unit (blue), ultraviolet filtration (purple), and two protein skimmers (green) supplied by
two mechanical pumps (black). The water quality was within acceptable standards for all parameters tested (pH = 7.70-8.20; salinity = 35 ppt; temperature = 26°C-28°C; ammonia = 0.00 mg/l; nitrite = 0.00 mg/l; nitrate = < 15 mg/l; Carlson, 1987; Barord and Basil, 2014). Each nautilus was fed either a shrimp with shell or lobster carapace every 4-5 days. The system was cleaned and maintained on a daily basis. We used a within-subject design to reduce the number of animals captured from the wild, and allowing for each animal to serve as its own control to control for individual variation.

Figure 2.1 Diagram of the Nautilus system with arrows denoting water flow and color codes corresponding to life support equipment including mechanical pumps (black), chilling unit (blue), ultraviolet filtration (purple), and protein skimmers (green).

2.2.2 Experimental Maze

We used one of the holding systems (tank 2 in Figure 2.1) as the experimental tank to contain the maze. A circular arena was constructed out of black acrylic and designed to fit securely into the holding tank, approximately 10 cm below the water surface (about shell depth) to create a shallow ‘inverse Morris water maze’ (Figure 2.2; Morris, 1984; Boal et al., 2000; Crook et al., 2009; Crook and Basil, 2013). A hole was cut along the periphery of the arena, to allow access to the deeper water of the tank (reward). A contrasting “beacon” was placed around
the hole during training (A black and white striped contrasting ring around the exit, covered in bubble wrap; Figure 2.2). The beacon served as the visual cue for the nautilus to move toward during the trials (as in Crook and Basil, 2008) and served as an associative cue for the goal. The criterion used in these studies was resting on the beacon with at least one tentacle touching the beacon, as the nautiluses showed a preference for the texture of the beacon over exiting the maze.

![Figure 2.2](image)

Figure 2.2 Excerpt from Crook and Basil (2013) showing experimental setup of maze used during the current spatial study. Figure by Robyn Crook.

Each day, in random order, the nautiluses (N=15) were trained to find the goal using the beacon over five trials, each lasting a maximum of 10 minutes, and then tested after one minute with one experimental probe trial (no beacon, 45° or 90° shift). A total of five nautiluses were then used for each testing/probe condition (no beacon probe, shifted 45° beacon probe, and
shifted 90° beacon probe). The arena and tank were surrounded by a curtain, to avoid external cues (Crook and Basil, 2013) with a video camera placed directly above (150cm) as in previous studies (i.e., Crook and Basil, 2008) and lighting was from normal fluorescent lighting from the room as well as an additional overhead incandescent lamp.

![Figure 2.3](image)

Figure 2.3 Training and three different probe conditions; 1a, training trial for beacon group, 1b, probe condition with 45° beacon, 1c, probe condition with 90° beacon, and 1d, Probe condition without beacon. S and arrow indicate start position of each animal.

2.2.3 Training Trials

All nautiluses were trained identically with a beacon present over five 10-minute trials. Each nautilus was placed at the start position with its tentacles facing right (arrow in Figure 2.3a). Next, the nautilus was released and the trial clock started. The trial was observed via an external monitor and if the nautilus attached to the maze and stopped moving, a plastic zip tie was used to gently disturb the nautilus so it would continue moving about the maze. The trial was ended when either (a) the animal successfully located the beacon by having at least one tentacle attached to it, or (b) ten minutes had elapsed with the nautilus not locating the beacon. During training, the nautilus explored the maze, and either 1) located the goal, indicated by the
beacon, and was rewarded with five minutes of time attached to the beacon (they preferred the texture of mesh over the exit), or 2) if they did not find the beacon, they were gently drawn to the beacon by allowing their tentacles to attach to the experimenter’s fingers and being guided to the goal (Crook et al., 2009). Nautiluses were still rewarded with the five minutes of time attached to the beacon. It is important to note here that the nautiluses were trained to locate the area designated by the beacon; this was their goal. Thus, when the beacon was removed or shifted, the goal was still to locate the area where the beacon was placed originally. Throughout this manuscript, ‘goal’ will be used to refer to this original position of where the beacon was placed.

2.2.4 Testing Trials

In between training and testing, the nautilus was removed from the maze and placed in a dark container for one minute. Then, a probe/test trial was performed by either 1) removing the beacon or 2) repositioning the beacon at a 45° or 3) 90° angle from the original beacon location. Here, we tested whether the nautilus focused on the beacon (beacon homing) or reverted to route memory to complete the trial. During the probe test, the nautilus was given a maximum of five minutes or five “disturbances” to make a positional choice, given the cue they had learned in training was shifted or absent. Their final positional choice was recorded along with the speed with which they settled in that position (latency).

2.2.5 Data Analyses

All trials were recorded via video camera for future analyses of total path distance, velocity, and overall movement patterns using Metamorph™ tracking software. Statistical analyses were performed using Microsoft Excel and SPSS. Repeated-measures ANOVA was used to determine the effect of the independent variables (training trial number, probe condition)
on the dependent factors (time to find goal, path length, etc.). A two-sample t-test was used to compare any post-hoc differences across conditions.

2.3 Results

2.3.1 Training Trials

The nautiluses learned to find the goal, designated by a beacon, quickly and consistently in each of the three probe-condition groups. There were no statistically significant differences in speed to find the beacon across training among the three different probe-treatment groups (Figure 2.4; means reported in Table 2.1); no beacon probe group training (F(4,364) = 2.093, p=0.081), 45° beacon probe group training (F(4,196) = 0.51, p=0.728), and 90° beacon probe group training (F(4,156) = 0.45°9, p=0.766).

Table 2.1 Mean trial latency for nautiluses in five training trials of three different conditions.

<table>
<thead>
<tr>
<th></th>
<th>No Beacon</th>
<th>Shifted 45</th>
<th>Shifted 90</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (s)</td>
<td>SD</td>
<td>Time (s)</td>
</tr>
<tr>
<td>Trial 1</td>
<td>166.85</td>
<td>111.36</td>
<td>Trial 1</td>
</tr>
<tr>
<td>Trial 2</td>
<td>156.55</td>
<td>133.70</td>
<td>Trial 2</td>
</tr>
<tr>
<td>Trial 3</td>
<td>166.29</td>
<td>121.57</td>
<td>Trial 3</td>
</tr>
<tr>
<td>Trial 4</td>
<td>142.06</td>
<td>90.91</td>
<td>Trial 4</td>
</tr>
<tr>
<td>Trial 5</td>
<td>174.44</td>
<td>176.66</td>
<td>Trial 5</td>
</tr>
</tbody>
</table>
2.3.2 Trial Five versus Probe Trials

To determine if shifting or removing the beacon altered the behavior of the nautiluses, we calculated the difference in time to complete a trial for a nautilus from trial 5 to the testing/probe condition (Figure 2.5), for each probe-condition group. We then used a nonparametric Mann-Whitney U test to determine if the changes in performance from the last training trial (5) to the probe were different for the 3 treatment groups. We did not find any statistically significant differences in how long it took nautiluses to solve the maze in the test trials compared with the final training trial. There was a trend for the beacon presence, even when shifted, to override the route information available for animals to find the goal. This is reflected in the longer latencies, as nautiluses would swim to the beacon first rather than the goal in these cases.
2.3.3 Testing Trials: Beacon Changes

The latency of only positive outcomes of the three different probe conditions were calculated (Figure 2.6). Here, there was a significant difference in the time taken for the nautiluses to complete the trial among the three different probe conditions (ANOVA, overall, \( F(2, 78) = 5.312, p=0.007 \); no beacon = 184.55s ± 10.77; shifted 45° probe = 114.60s ± 11.65; shifted 90° probe = 150.58s ± 13.05). Post-hoc analyses showed that there was a statistically significant difference in latency between the shifted 45° beacon probe and the no beacon probe trials (\( p=0.006 \); no beacon = 184.55s ± 10.77 vs. shifted 45° probe = 114.60s ± 11.65) but no difference between the shifted 45° probe and the shifted 90° probe group (post hoc t-test; \( p=0.128 \); shifted 45° probe = 114.60s ± 11.65 vs. shifted 90° probe = 150.58 ± 13.05). There was
no statistically significant difference between the no beacon probe and the 90° beacon probe tests (p=0.761; no beacon = 184.55s ± 10.77 vs. shifted 90° probe = 150.58s ± 13.05).

The percentage of nautiluses finding the exit hole versus choosing the shifted beacon across test conditions was then calculated (Figure 2.7). There was a significant difference in the probe trial outcomes of the nautiluses’ choice during navigation depending upon probe-condition. (ANOVA, overall, F (4, 20) = 12.818, p=0.000). When the beacon was removed during probe trials, the nautiluses located the hole 70.00% of the time which was a significantly greater rate than when the beacon was shifted 45° (p=0.001) or shifted 90° (p=0.006). When the beacon was shifted 45° the nautiluses selected the beacon (53%) more times than the hole (40%; p=0.036). Likewise, when the beacon was shifted 90°, there was also a significant difference between the nautiluses’ choice of navigating to the beacon or the hole. In these cases, however,
the nautiluses selected the hole (50%) significantly more often than the beacon (33%; p=0.021). When the beacon was shifted, in other words, the nautiluses located the hole on significantly fewer times when the beacon was shifted 45° compared to the shifted 90° beacon (p=0.010). In general the presence of a beacon over-rode route memory, unless no beacon at all was present.

![Average Outcomes During Probe Condition](image)

**Figure 2.7** Percentage of nautiluses choosing the goal (hole) or beacon in probe tests. Black bars denote nautiluses that located the area where the beacon was previously located and the gray bars denote nautiluses that located the new position of the beacon. Error bars denote SEM.

Next, we analyzed the paths taken of the nautiluses during the training and probe trials using Metamorph tracking software. Here we show illustrative examples of the paths animals took during training and testing in the different probe conditions (Figure 2.8). By looking at the paths of nautiluses in trial one versus trial five (Figure 2.9), the linearity of paths, and turns taken while homing to the beacon can be visualized.
Figure 2.8 Navigational paths of nautiluses for each of the four different probe-test configurations; (a) training trial with beacon, (b) probe condition with beacon removed, (c) probe condition with beacon shifted 45°, and (d) probe condition with beacon shifted 90°.

Figure 2.9 Nautilus navigational path in training trial one (a) versus training trial five (b).
Next, we looked at the distance traveled by the nautiluses during the training and probe trials. There were no significant differences in distance traveled across the five training trials in within any of the three probe condition groups: no beacon group (ANOVA, overall, F (4, 438) = 1.924, p=0.105), shifted 45° beacon group (ANOVA, overall, F (4, 244) = 0.765, p=0.549; mean averages reported in Table 2.2), and shifted 90° beacon group (ANOVA, overall, F (4, 190) = 0.247, p=0.911). However, similar to latency, there was a general trend of decreasing distance traveled as trials continued (Figure 2.10). When we take the last training trial alone and compare the distance traveled during this trial to the probe test in the different conditions (Figure 2.11), the nautiluses did not exhibit different distances between the training and probe trials (ANOVA, overall, F (2, 185) = 1.554, p=0.214; no beacon - 5.011 ± 4.910, shifted 45° - 5.985 ± 5.159, shifted 90° - 4.304 ± 3.874). Lastly, during the probe conditions, overall the nautiluses traveled significantly longer distances (ANOVA, overall, F (2, 173) = 3.809, p=0.024) when the beacon was removed (p=0.007; 5.22m ± 0.410) or shifted 90° (p=0.030; 5.26m ± 0.720) than when the beacon was shifted 45° (p=0.960; 3.50 ± 0.434) from the original position (Figure 2.12).

Table 2.2 Means of total distance traveled during training trials in each of the different conditions.

<table>
<thead>
<tr>
<th>No Beacon</th>
<th>Shifted 45</th>
<th>Shifted 90</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distance (m)</td>
<td>SD</td>
</tr>
<tr>
<td>Trial 1</td>
<td>8.218</td>
<td>5.870</td>
</tr>
<tr>
<td>Trial 2</td>
<td>6.319</td>
<td>6.061</td>
</tr>
<tr>
<td>Trial 3</td>
<td>6.609</td>
<td>5.909</td>
</tr>
<tr>
<td>Trial 5</td>
<td>5.906</td>
<td>6.871</td>
</tr>
</tbody>
</table>
Figure 2.10 Total distance traveled during the five training trials of nautiluses in each of the different conditions. Error bars denote SEM.

Figure 2.11 Average change in distance traveled of *Nautilus* between the last training trial and the probe condition. Error bars denote SEM.
2.4 Discussion

Nautiluses perform daily vertical migrations (Carlson et al., 1984; Ward et al., 1984; O’Dor et al., 1993) across coral-reef slopes that were believed to represent migration patterns in all nautiluses. However, recent evidence from a population of nautiluses in Osprey Reef, Australia suggests that daily migration may be more complex and variable (Dunstan et al., 2011). Although the reasons for these differences are misunderstood, the variability may be explained by a number of factors, including local topography, variation in the different populations, or perhaps species composition. Here, we closely examined the role topography and/or route memory plays in nautilus homing.

We found that nautiluses could learn to find a goal both using beacon information and route memory. This is the first demonstration of route memory in nautiluses. When a beacon is removed during testing, nautiluses still find the exit goal 70% of the time, implying they
simultaneously learn both the route memory/direction and the visual beacon during training (Gibson and Shettleworth, 2005). However, if a beacon is present during a test, the nautiluses fail to rely on their route memory in favor of the beacon, as was evidenced by their longer path lengths and slower speed when the beacon was absent. This implies overshadowing of route memory by the beacon (Rescorla and Wagner, 1972) or the nautiluses may be relying on directional memory only, rather than route memory.

This suggests two interesting features of nautilus homing: 1) they can use route memory or potentially directional memory in the absence of a cue to solve a problem and 2) the presence of a beacon in testing overshadowed route and directional memory. Nautiluses were more likely to choose the beacon over the goal, resulting in their longer latencies and longer path lengths when the beacon was absent. Based upon path analysis, nautiluses would swim toward the beacon first, then only later move toward the outlet goal. These results suggest that the nautiluses are able to switch navigational tactics depending on their environmental cues, but that the cues are weighted differently in memory. This is in keeping with the idea that simultaneously learned sources of information compete for memory space (Rescorla and Wagner, 1972). An alternative explanation during the no beacon tests may be that nautiluses are relying on directional information alone, rather than memory for the entire route. Nautiluses may have found the goal by recalling only the direction, and not the distance, to the goal -- an alternative to route/kinesthetic memory.

The placement of the beacon affected the nautiluses’ ability to find the exit (Figure 3.6). When the beacon was removed, the nautiluses still found the exit 70% of the time, supporting that the nautiluses switch to route or directional memory when the visual cue is removed. When we look at the success rate of finding the goal when the beacon was shifted (i.e., locating the
original position of where the beacon used to be), the nautiluses successfully completed the maze 40% in the 45° shifted condition and 50% of the time in the 90° shifted condition. That nautiluses found the exit more quickly when the beacon is shifted 90° versus 45° indicates that the greater the beacon shift, and mismatch with route memory, the less salient the beacon becomes and the nautiluses may revert back to route memory.

While altering the landscape (removing or shifting the beacon) appears to change the navigational tactic used, the nautiluses still complete test trials at similar speeds to the fifth training trial. However, upon closer examination, this was due to the nautiluses at first homing in on the beacon and then traveling to the goal. Thus their swimming path, rather than speed to locate the goal, is probably a more accurate measure of their learning and remembering.

If the beacon is gone, the nautiluses revert to route or directional memory. If the beacon is shifted 45°, the nautiluses use beacon homing first to find their goal, then shift when it is incorrect. If beacon and route memory are in greater conflict (the 90° condition) animals are more likely to ignore the beacon and revert to route/directional memory. When the beacon was removed or shifted 90° (becoming less reliable), the nautiluses traveled significantly greater distances to the goal (Figure 2.12) and suggests that the nautiluses were reverting to route memory during these probe trials only after searching for the beacon. The learning of the beacon and goal is also evident when we look at the paths taken by animals in the first trial of training versus the last training trial, and then the subsequent probe trials (Figure 2.8 and Figure 2.9). Here, the nautiluses are more likely to explore more areas of the arena in the first trial than in trial five or the probe trials (Figure 2.9). Their homing is more directed with training and experience whether the beacon has moved in testing or not. Thus, these animals can dynamically switch between simultaneously learned tactics depending upon changes to their environment.
Previous studies suggested that nautiluses may navigate their natural habitat by using a variety of visual cues and the three dimensional layout of their environment (Crook et al., 2009; Basil et al., 2012; Crook and Basil, 2013; Basil and Crook, 2014). We demonstrate here that they can also use route or direction memory to find a goal, especially when a visual cue is unreliable. The apparent overshadowing of the beacon over route memory may imply that visual cues are more important in their natural environment than previously thought, given their lack of acute vision (i.e., Muntz, 1987). Perhaps the visual cues are related to sites for reproduction, or hiding spots from predators, two vitally fitness-related aspects of their daily lives. The ability to switch navigational tactics when the visual cue becomes less reliable would also be a beneficial adaptation. As the potential visual cues become less reliable, the nautiluses can ‘fall back’ to using route/direction memory to navigate their environment. This flexibility in homing provides a clear comparison to the coleoids and informs our understanding of behavioral complexity in the lineage as a whole.

Future investigations into potential topographical features of their natural habitat will help determine habitat types and components of each habitat that are important to nautiluses (perhaps using BRUVS). These features and habitats could then be managed in the wild to provide protected areas that may be more supportive of nautiluses. In addition, determining if certain topography in the wild is associated with good foraging locations, egg deposition sites, or hiding spots will inform management efforts, now that we know nautiluses do learn and return to distinct spatial locations. Protecting those locations that nautiluses prefer and can remember is vital to their preservation.
Chapter 3. Foraging and Scavenging Behavior in *Nautilus*: A Field and Laboratory Study

3.1 Introduction

An organism’s ability to successfully forage and the content of their diet both play a vital role in every aspect of their life history. The deep sea habitat of nautiluses, until recently, has prohibited direct observations of their feeding habits in the wild. In addition, baited traps used to catch nautiluses may also lure non-traditional prey items into the trap, which the nautilus may then consume opportunistically. It is thus difficult to observe their natural foraging behavior. We know nautiluses consume a variety of crustaceans (particularly hermit crabs in some cases; Ward and Wickstein, 1980), arthropods, and fishes based upon gut content analyses (Ward and Wickstein, 1980) and aquarium observations (Carlson, 1987). In captivity, nautiluses are fed and will eat a variety of different food items from frozen shrimp with shell (assuming that the shell contains calcium required for growth), crustacean molts, fishes, and in at least one case, live crab and live shrimp (Basil, pers. obs). While hermit crabs were found in the gut of nautiluses in New Caledonia (Ward and Wickstein, 1980), live hermit crabs placed in the same enclosure with nautiluses were not preyed upon and simply became co-inhabitants of the system with the nautiluses (Barord Unpub, 2008).

Most cephalopods are active predators in the wild (see review - Boucaud-Camou and Boucher-Rodoni, 1983; Castro, 1991; Mangold et al., 1993; Budelmann, 1996; Oestmann, 1997; Markaida et al., 2003; Hanlon and Messenger, 2010). The coleoid cephalopods, octopuses squid, and cuttlefish, rely on vision, camouflage, and either 8 arms (octopuses) or 8 arms-2 tentacles (squid and cuttlefish) to ambush their live prey. On the other hand, nautiluses (Subclass Nautiloidea) have poor vision, lack chromatophores, and have up to 90 tentacles that lack suckers. The tentacles are enclosed in buccal sheaths and can either be retracted (unexcited or
defensive behavior) into the buccal sheaths or protracted (excited) from the buccal sheaths (Bidder, 1962; Kier, 1987; Basil et al., 2000; Ruth et al., 2002; Basil et al., 2005). The assumption has been that all cephalopods were active predators, nautiluses included, until recent work suggested that the vampire squid was in fact a detritus feeder (Hoving and Robinson, 2012). Nautiluses have been described as “smellers and gropers” (Saunders, 1984): predators and scavengers opportunistically feeding on a variety of different prey items (Ward and Wickstein, 190°8; Carlson et al., 1984; Saunders 1984; Ward et al., 1984; Hayaska et al., 1987; Saunders and Landman, 1987; Saunders and Ward, 1987; Ward, 1987; Wells et al., 1992). However, no direct evidence is available to either confirm or refute these claims.

There is also the question of how nautiluses locate, catch, and consume prey items in the wild. If nautiluses are active predators, how do they first locate their prey item? If nautiluses are scavengers, how do they locate prey and what type of prey do they search out? We know nautiluses can track odor from a distance of at least 10m (Basil et al., 2000) and use a combination of their rhinophores at a distance and tentacles in proximity to locate the odor source precisely (Basil et al., 2000; 2005). As nautiluses search for food, their tentacles spread laterally, vertically, and dorsally, a ‘cone of search’ posture, which expands their search area relative to their body (Bidder, 1962; Boucher-Rodoni, 1983; Basil et al., 2000; Basil et al., 2005). When a prey item is located usually by the long digital tentacles dragging near the substrate, the medial tentacles bring the food to the mouth (Bidder, 1962; Basil et al., 2005). It appears that there are both chemical and tactile cues used in Nautilus when searching for food (Haven, 1972; Ruth et al., 2002; Basil et al., 2000; 2005).

Although nautiluses live in a deep sea environment with nominal to no light, they may also rely on vision to either (a) navigate their reef environment (Crook et al, 2009) or (b) locate
bioluminescent prey items organisms, as they are positively phototactic (Muntz and Raj, 1984; Muntz, 1986; Muntz, 1987). The Nautilus eye has been of interest and studied for nearly two centuries (Owen, 1832; Hensen, 1865; Griffin, 1897; Mugglin, 1937; Young, 1965; Barber and Wright, 1969). Muntz and Ray (1984) designed an elegant experiment to determine the effectiveness of the visual system in Nautilus. Overall, they suggested that the pin-hole camera eye with no lens in Nautilus produced lower resolution, decreased sensitivity, and a narrowed field of view, all resulting in poor vision. However, the authors did find that the Nautilus retina was similar to the retina in Octopus (Young, 1962), with up to 4 million photoreceptors tuned to wavelengths of 470nm (blue) (Muntz and Raj, 1984; Muntz, 1986; 1987) and may suggest an affinity to locate light (such as bioluminescence, also within the 470nm range) from long distances. Given the limitations of the Nautilus visual system, Muntz and Raj (1984) still conclude that vision is indeed important for Nautilus, given some of the well differentiated structures of the eye. Finally, recent studies provide further evidence that nautiluses use vision to locate and remember variety of visual landmarks in a maze (Crook et al., 2009; Crook and Basil, 2012). Thus vision is perhaps more important to nautiluses than previously thought.

Recent field work using baited remote underwater video systems (BRUVS) to document the abundance of nautiluses throughout the South Pacific (Barord et al., 2014) suggests that nautiluses are not only scavengers of dead prey items, but are strict scavengers of dead prey items with no inclination to catch and consume live prey items. Here, we describe the foraging of nautiluses in the wild, from BRUVS footage. These observations are paired with controlled laboratory tests of their foraging behavior in a semi-naturalistic tank, so we could directly measure 1) if and how they detect buried prey, 2) their tracking behavior to locate the prey from
Understanding the diet and foraging habits of nautiluses is critical to their care in captivity and to our understanding of their life history in the wild. The diet of an organism can be determined in many ways -- from direct observations to stomach content sampling and fecal analysis. Addressing the questions surrounding the feeding habits of nautiluses would not only improve husbandry conditions and therefore, research practices, but also support future conservation efforts to protect the species from overexploitation and extinction (DeAngelis, 2012). Here, we apply field and laboratory observations to describe how nautiluses locate and capture potential prey items.

3.2 Materials and Methods

3.2.1 Field Trials

Baited remote underwater video systems (BRUVS) were deployed during population surveys at four different geographic regions: Philippines, Australia, Fiji, and American Samoa (Table 3.1; Barord et al., 2014). Each BRUVS unit consists of one HD camcorder and LED light, each enclosed in an underwater housing and mounted to a steel frame (Figure 3.1). A bait stick extends from the frame in view of the camera and rope leads up to a buoy at the surface. The BRUVS are baited with chicken and deployed at dusk, before sunset, and retrieved the following morning at dawn resulting in an approximate deployment or ‘soak’ time of 12 hours. The BRUVS are deployed between depths of 300-400m, depending upon the local topography in the habitat that nautiluses are commonly caught (Dunstan et al., 2011) so the footage should provide the most accurate depiction of nautilus population abundance and their behaviors. These depths
are similar depths used by nautilus fishermen so the likelihood of capturing numerous nautiluses on film successfully is high. Three BRUVS were deployed at the Australia site each night for nine total nights. Two BRUVS were deployed per night in the Philippines (five total deployment nights), Fiji (five total deployment nights), and American Samoa (five total deployment nights).

Upon retrieval, the video was downloaded to portable hard drives for later analysis of the footage. Here, analysis of the footage includes 1) documentation and description of foraging behaviors of nautiluses attracted to the bait, as well as 2) identifying potential live prey items of nautiluses.

<table>
<thead>
<tr>
<th>Site</th>
<th>Number of Nautiluses</th>
<th>Number of Nautiluses Foraging</th>
<th>Hours of Footage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Philippines</td>
<td>6</td>
<td>1</td>
<td>150</td>
</tr>
<tr>
<td>Australia</td>
<td>92</td>
<td>5</td>
<td>190</td>
</tr>
<tr>
<td>Fiji</td>
<td>20</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>American Samoa</td>
<td>22</td>
<td>4</td>
<td>120</td>
</tr>
</tbody>
</table>
Figure 3.1 Schematic of baited remote underwater video systems (BRUVS) frame deployed to record video footage of nautiluses at depth. The LED light (L) and HD camcorder (C) housings were fastened securely to the mounting brackets shown above. The bait (B) was placed on the end of a $\frac{1}{2}'$ PVC stick extended 50cm from the BRUVS frame.

3.2.2 Semi-naturalistic Foraging Trials: Husbandry

Maintaining nautiluses in captivity requires excellent water quality, specialized equipment, and consistent observations. The microvillus epidermis of nautiluses, and all cephalopods, results in a greater uptake potential of toxins in the water. The relatively high metabolism of nautiluses means that nitrogenous waste products will build up in the system more quickly. With a porous epidermis, high metabolism, and significantly high sensitivity to any metal concentration in the water (especially Cu$^{+2}$), efficient life support systems are needed (Carlson, 1987; Barord and Basil, 2014). Water quality must be maintained through biological, mechanical, and chemical filtration. Effective filtration will convert the nitrogen products into
less toxic by-products such as nitrite and nitrate, nitrate being the least toxic to cephalopods.

While there are filtration methods to remove nitrates, i.e., denitrification units, consistent water changes that remove the nitrates are acceptable and also provide other intrinsic benefits when changing out water. While there has been increasing literature on nautilus veterinary treatments (Barord and Henderson, 2008; Scimeca, 2012; Barord et al., 2012; Barord and Basil, 2014), this is an area still misunderstood. So, monitoring of health issues is critical so they are noted as soon as possible and treatments can be employed quickly. Maintaining a high quality of nautilus health ensures that the nautiluses have the ability to behave normally and without issue.

Wild caught *Nautilus pompilius* from the Philippines and supplied by SeaDwelling Creatures Inc were used in the semi-naturalistic scavenging study in Brooklyn. All of the animals were housed in a 2625 l recirculating sea-water system (Figure 3.2), kept in dim light and at 14-17 degrees C to simulate their deep-water natural environment. The system included three cylindrical holding tanks (1.5 m tall, 1 m diameter), a 187.5 l sump holding biological filtration, a mechanical pump (black), a chilling unit (blue), ultraviolet filtration (purple), and two protein skimmers (green) supplied by two mechanical pumps (black). Six nautiluses were contained in each tank to avoid overcrowding. Water quality and animal health were monitored on a daily basis (pH: 7.70-8.30, Temperature: 16 °C-18 °C, salinity: 35 ppt, NH₃: 0.00 mg/l, NO₂: 0.00 mg/l, NO₃: <15 mg/l). The water quality was within acceptable standards for all parameters tested (Carlson, 1987; Barord and Basil, 2014) throughout the experiments. Each animal was fed either a shrimp with shell or lobster carapace every 4-5 days. The system was cleaned and maintained on a daily basis. Animals were checked for their state of health at least 2 times a day.
3.2.3 Semi-naturalistic Foraging Trials: Tank Setup

The foraging tank was a rectangular (140x38x30 cm), recirculating aquarium with 5cm of sand substrate placed on the bottom (Figure 3.3). Flow was laminar from the upstream portion (point ‘U’) to the downstream area (point “D”). The aquarium (167 L) was enclosed by a dark curtain surrounding the sides of the tank, only allowing ambient light to come in from above. Within the curtains, two video cameras (Panasonic HC-V720) were placed in 1) a permanent position facing the intake (camera a, Figure 3.3 and Figure 3.4) or a 2) position at a semi-random quadrant where prey items were placed during testing (camera b, Figure 3.3 and Figure 3.4). This provided a view upstream from the foraging animal, and a close (camera b) view of the animal at the foraging site. A TV monitor (15” COBY® LED TV1526) was connected to each video camera so the animal could be observed from outside the curtains. The water quality conditions of the experimental setup were maintained in the same manner as the permanent Nautilus holding aquarium system. Each nautilus (N=10) experienced three phases during the experiment: habituation, training, and testing.
Figure 3.3 Side view of scavenging setup showing water flow (arrows) with the starting position, S, in front of the intake pipe and the camera, a, facing the intake. Flow was laminar in the tank from point U to point D.

3.2.4 Semi-naturalistic Foraging Trials: Habituation

During habituation, each nautilus was acclimated to the setup for 20 minutes per day, over three days. Here the nautiluses were given an opportunity freely explore the experimental tank for 20 minutes. To elicit a positive olfactory response in the tank, a tuna slurry concentrate was mixed by adding the contents of a 142g can of tuna to 1 liter of artificial seawater and homogenizing, similar to the method employed by Basil et al., (2000). 0.5 ml of the slurry was then pipetted over the tentacles of the nautiluses at five-minute increments during the 20-min habituation period. The aim was to create an association to find/explore for food in the tank. Care was taken to monitor for any stress behaviors the animals might exhibit (hyperventilation,
“rocking behavior”) in the novel tank. The animals did not exhibit any stress behaviors and swam freely in the tank.

3.2.5 Semi-naturalistic Foraging Trials: Training

During training, a tuna-slurry-soaked shrimp was semi-randomly placed on the surface of the sand in one of four quadrants (Figure 3.4) downstream from the inlet, to carry the odor to the animal’s start position (as in Basil et al., 2000) further downstream (confirmed with dye tests). The animal was then placed in the “start” position and recording commenced. Here we were training the animals that food was available in the tank, and also documenting if the animals had to learn to find the food item, or naturally were able to track it (either visually or using odor in this stage of the study).

Figure 3.4 Top view of foraging tank showing the four semi-random quadrants in which the shrimp was placed for training and testing trials. Upstream is left and water flowed downstream to the outlet pipe on the right (arrow). The start position, S, is near the outlet pipe. Nautiluses were placed there, tentacles forward as in Basil et al (2000; 2005) to ensure contact with the odor plume emanating from the shrimp. A video camera, a, was positioned facing the start position from upstream every trial while video camera b, was positioned at the quadrant with the shrimp. The entire setup was enclosed with a curtain (outer box) and monitored from the outside to avoid stressing the animal.
Here we trained the animals that food could be found at different locations in the flow-through foraging tank (locations that would later become possible sites for a buried item during testing). Animals (N=10) were given 3 training trials, 1 per day, over the course of 4 days. Each trial lasted 20 minutes from when the animal was placed in the “start” position. The entire training trial was recorded, and success rate in locating the surface shrimp was calculated across days. Criterion was met when the animal obtained the shrimp in 2/3 days in a row.

3.2.6 Semi-naturalistic Foraging Trials: Testing

During testing, one tuna-scented shrimp was randomly buried (1cm below the surface of the substrate) in one of the four quadrants in the foraging tank to mimic conditions a scavenger in the wild might encounter. Cameras and monitors were as above. The nautilus was placed in the start position. Again, the nautilus was given 20 minutes to locate the buried shrimp, or to end the trial. Each animal was tested once per day, until they successfully found the buried prey item in 3 out of 4 days. The video from each trial was analyzed to describe and measure behaviors such as, path to prey, latency to find prey, searching posture during odor tracking, and tentacle use and funnel movement at the prey location.

3.3 Results

3.3.1 Foraging/Scavenging Behavior in the Wild

The baited remote underwater video systems (BRUVS) recorded over 400 hours of underwater footage from the four survey sites (Table 3.1). A greater number of nautiluses was recorded at sites without historical nautilus fisheries (Australia, Fiji, and American Samoa) versus the site with historical nautilus fisheries (Philippines; Barord et al., 2014). Although a total of 148 nautiluses were recorded via the BRUVS, only 13 nautiluses were recorded that
exhibited foraging or scavenging behaviors (Table 3.1). The 13 nautiluses behaved in a similar manner to those described in controlled odor-tracking studies (Basil et al., 2000; Westermann and Beuerlein, 2005; Basil et al., 2005). Specifically, nautiluses exhibited the classic “cone of search” posture (Figure 3.5 and Figure 3.6; Bidder, 1962; Basil et al., 2000; Basil et al., 2005), extending tentacles and swimming slowly (>5cm/s) with tentacles forward (nautiluses naturally often swim shell forward otherwise to reduce drag). All 13 of the nautiluses displayed the cone of search behavior from a distance of 0.5 to at least 5 m from the camera. In two cases, nautiluses clearly adopted the cone of search posture 5m away from the bait source. From 5 m away, the nautiluses slowly jetted toward the bait, tentacles first, while turning side to side (Figure 3.7), as in Bidder (1962) and Basil et al. (2000).

Figure 3.5 Nautilus pompilius performing the ‘cone of search’ behavior directed towards a bait source (chicken in red mesh) recorded from BRUVS observations along the Great Barrier Reef, Australia. Photograph inset with close-up of nautilus with digital tentacles (dt) and digital lateral tentacles (dlt) extended and hyponome (h) jetting nautilus along substrate.
Figure 3.6 *Nautilus pompilius* performing the ‘cone of search’ behavior directed toward (change all towards to ‘toward’) a bait source (chicken in red mesh) recorded from BRUVS along the Great Barrier Reef, Australia. Photograph inset with close-up of nautilus showing digital tentacles (dt) and digital lateral tentacles (dlt) spread out and hyponome (h) jetting nautilus along substrate.
Lastly, the BRUVS also recorded interactions between nautiluses and potential live prey items. The greatest number of interactions was between the nautiluses and various species of shrimp, presumably *Heterocarpus* sp. (Figure 3.8 and Figure 3.9). One interaction was recorded between a single juvenile *N. pompilius* and a hermit crab (presumably *Pagurus* sp.). At no point did any of the nautiluses display predatory behaviors toward any of the live prey items. Instead animals directed odor-tracking behaviors toward the dead bait – supporting the notion they are primarily scavengers in the wild.
Figure 3.8 *Nautilus pompilius* feeding on the bait (chicken in green mesh) with several shrimp, *Heterocarpus*, crawling over the nautilus. Footage from BRUVS in Panglao, Philippines.

Figure 3.9 *Nautilus pompilius* searching for bait source (canned fish) in Taena Bank, American Samoa. Note three shrimp (possibly *Heterocarpus* sp.) crawling on its hood and shell. Lateral digital tentacles are extended.
3.3.2 Foraging/Scavenging Behaviors in Semi-naturalistic Conditions - Habituation

During habituation, the nautiluses explored the entirety of the tank over the 20 minute trial, often coming to rest attached to the side of the tank with their tentacles. They did not exhibit any stress behaviors. When the tuna concentrate was pipetted over the nautiluses’ digital tentacles, each of the nautiluses extended its tentacles outward and jetted around the tank in the classic cone of search posture.

3.3.3 Foraging/Scavenging Behaviors in Semi-naturalistic Conditions - Training

Prior the 0.5ml of tuna stimulus being injected, the nautiluses remained partially to fully closed in their shell with the hood closed and their tentacles retracted into the buccal sheaths (Figure 3.11a). After pipetting the tuna slurry across the tentacles, the nautiluses immediately started to open up their shell and project their digital tentacles (Figure 3.11b). With the hood open, the pre-ocular and post-ocular tentacles were now visible. The nautiluses then extended their digital tentacles and digital lateral tentacles in the cone of search behavior (Figure 3.11c). During the search phase, the nautiluses continued extending their tentacles and also dragged their digital lateral tentacles across the substrate. During these trials, the shrimp was placed on the surface so when one lateral digital tentacle touched the shrimp during searching, the medial digital tentacles were immediately directed towards the shrimp and it was taken up quickly as the nautiluses jetted away (Figure 3.11d).
Figure 3.11 Foraging and scavenging behaviors of *Nautilus pompilius* during training trials when the shrimp was placed on the surface. Pre-scent behavior characterized by an overall retraction of most, if not all tentacles (a); post-scent behaviors include extending digital tentacles (dt) and opening up of the hood to expose the post-ocular (pooc) and pre-ocular (proc) tentacles (b); active searching for food characterized by a “cone of search” with digital and digital lateral tentacles (dlt) extended outward with hyponome (h) propelling and possibly disturbing sediment during search (c), and finally, successful location of the prey item and grasping of the shrimp with its medial digital tentacles (d).

Training: Of the 10 nautiluses, seven reached the success criterion of locating the shrimp at least two out of three times across days. Five of the nautiluses located the shrimp in all three trials, three found the shrimp two out of three times, and one located the shrimp one out of three times. A single nautilus did not locate the shrimp on any attempts although it displayed similar
search behaviors to each of the other nautiluses. This could be due to a failure to find the item, or lack of motivation (unlikely, given the posture of the animal). They did not appear to improve in their accuracy over trials (no statistical difference between successful shrimp finding across the training trials ($X^2(2) = 0.341, p=.843$)). Also, animals did not find the shrimp more quickly with experience. There were no statistically significant differences in latency to find the shrimp among the three training trials (Figure 4.10; analysis of variance with repeated measures: $F(2,18) = 3.376, p=0.057$).

![Latency to Locate Shrimp during Training Trials](image)

Figure 3.10 Latency of *Nautilus pompilius* to locate the shrimp during training trials when the shrimp was placed on the surface of the substrate. Error bars denote SEM.

**Surface Tests:** When at rest the tentacles of the nautiluses were retracted into the buccal sheaths and the hood was partially to fully closed (Figure 3.12a). Directly after odor detection, the hood opened up and the tentacles extended out of the buccal sheaths (Figure 3.12b). Also visible at this point were the pre-ocular and post-ocular tentacles. Next, the nautilus extended its digital tentacles and digital lateral tentacles outward in the *cone of search* posture and jetted around the tank (Figure 3.12c). During this search behavior, the digital lateral tentacles were also
dragged along the substrate. Finally, when the nautilus located shrimp (or the area where the shrimp was buried during testing trials) the nautilus either used its medial digital tentacles to grab the shrimp (during training trials) or used a combination of its digital tentacles, lateral tentacles, and hyponome to dig in the substrate in search of the prey item (Figure 3.12d).

Figure 3.12 Foraging and scavenging behaviors of *Nautilus pompilius* during testing trials at four different stages during buried shrimp location in foraging tank. Pre-scent behavior characterized by an overall retraction of most, if not all tentacles (a); post-scent behaviors include extending digital tentacles (dt) and opening up of the hood to expose the post-ocular (pooc) and pre-ocular (proc) tentacles (b); active searching for food characterized by a “cone of search” with digital and digital lateral tentacles (dlt) extended outward with hyponome (h) propelling and possibly disturbing sediment during search (c), and finally, successful location of the prey item and subsequent digging behaviors (d).
3.3.4 Foraging/Scavenging Behaviors in Semi-naturalistic Conditions - Testing

During testing, a tuna soaked shrimp was buried 1 cm below the substrate and each nautilus was provided with four attempts to locate the prey item. Here, the criterion for success of each nautilus was locating the shrimp at least three out of four times. There were no statistically significant differences in success rates of locating the shrimp across the different training trials ($X^2(3) = 3.581$, $p = .310$; Trial 1 = 681.60 ± 151.96; Trial 2 = 1090.20 ± 80.90; Trial 3 = 856.90 ± 146.59; Trial 4 = 928.70 ± 120.22). Only two of the nautilus located the shrimp on all four trials. These same two nautilus therefore met the criterion of $\frac{3}{4}$ successful trials. Three nautilus located the shrimp in two out of four trials. Four nautilus located the shrimp one out of four times and a single nautilus did not locate the shrimp on any attempts. It should be noted here that the nautilus that did not locate the shrimp during training was not the same nautilus that did not locate it during testing. Animals did appear to find the buried item at different rates with experience (Friedman’s Test ($X^2(2) = 14.538$, $p = 0.002$), but pairwise comparisons (SPSS, 2012) with a Bonferroni correction for multiple comparisons did reveal an overall increase in speed to solve the problem reveal (Figure 3.11) across trials.
3.5 Discussion

Here, we provide evidence that nautiluses prefer dead prey items rather than live prey items, supporting our hypothesis that nautiluses are primarily scavengers (i.e., smellers and gropers; Saunders, 1984). We also documented foraging and scavenging behaviors in both the wild and in captivity and that these behaviors are remarkably similar in the different environments. Nautiluses are slow in their approach to dead prey items. This is in stark contrast to their extant relatives that rely on stealth, speed, and agility to capture live prey items.

The BRUVS were deployed to four separate survey sites across the South Pacific: Australia, Fiji, American Samoa, and the Philippines. The relatively small number of nautiluses recorded that exhibited scavenging behaviors (13) compared to the total number of nautiluses recorded (148) is an interesting result. An obvious bias was the limited field of view of the video camera that was only positioned in one direction. Potentially, nautiluses outside the initial view
of the camera may have been exhibiting the scavenging behaviors as they searched for the bait source.

The *cone of search* behavior was exhibited by each of the 13 foraging nautiluses. The *cone of search* and tracking behavior provides evidence that either (a) the nautiluses located the bait’s odor plume from far away or (b) that nautiluses are always in a constant state of arousal for scavenging. The fact that the bait was illuminated by a light and nautiluses are positively phototactic may have aided in their tracking, yet they still adopted the slow, back and forth tracking approach, with tentacles extended in the *cone of search*, despite the bright light. This supports the idea that olfaction in the wild is of crucial importance to nautiluses, whether other information is provided by the prey or not. In fact, the *cone of search* may be an innate or a fixed action pattern of behavior in response to odor for an animal that must opportunistically find food in a complex environment. Nautiluses do perform long vertical and horizontal migrations and appear to have short rest periods (Dunstan et al., 2011), -- it may be that scavenging in locations with access to food may affect their migrational patterns, as the *cone of search* seems to be so easily elicited.

Nautiluses are able to detect olfactory cues from far distances as evidenced by both laboratory study (Basil et al., 2000; Basil et al., 2005) and field study here. Two nautiluses exhibited the *cone of search* behavior at least 5m away from the bait source. Under laboratory conditions, nautiluses can detect odor plumes from over 10 m away (Basil et al. 2000), which supports the idea that the nautiluses in the wild may have been tracking the bait long before they entered the view of the camera. An obvious question would be how far away the nautiluses were able to detect the olfactory cue and eventually locate it. Given their affinity to locate odor and baited traps (Basil et al. 2000; Basil et al. 2005; Barord et al. 2014) and their long horizontal and
vertical migration abilities (Dunstan et al. 2011), the distance traveled to find a reliable olfactory cue may be great.

Another tracking behavior observed in these two nautiluses was their sinusoidal movements towards the bait source which appeared to get smaller and smaller as it approached the bait. This side to side approach that becomes increasingly more focused with proximity to the odor sources matches the behavior of nautiluses in flume studies (Basil et al., 2000). From the observations, we can make a representation of what these behaviors may look like (Figure 3.12). At greater distances from the bait source, the nautiluses were moving in a larger sinusoidal curve with the shell forward (Figure 3.12a). This would make sense when scavenging for prey items over great distances by (1) reducing drag and increasing jetting efficiency with shell forward and (2) by reducing predation by projecting the strong shell forward. This too was observed in nautiluses tracking in a flume (Basil et al., 2000). At a certain distance, perhaps between 5-15 m (Basil et al. 2000), the nautiluses turned 180° and began to move in smaller sinusoidal patterns with their tentacles forward and in the *cone of search* posture. When the olfactory cues suggest that the prey items are close, it would benefit the nautilus to begin searching with its many tentacles and to begin feeling along the substrate for prey as seen in Basil et al, (2000; 2005) and our current laboratory study.
In most cases, raw chicken was used as the potential prey item (i.e., bait source). In the instances when chicken was not available, canned tuna and mackerel was used. Regardless, the different baits elicited the same behaviors. Nautiluses approached the bait along the substrate with their tentacles extended outward and the hyponome propelling the nautilus along the substrate to the bait (“cone of search” as described in Bidder, 1962; Basil et al 2000, Basil et al 2005, Westermann and Beuerlein, 2005). Based on these observations, the hyponome may function both in movement and possibly for excavating the substrate, via water jets, for potential prey items. In at least 18 cases, we observed excavation of the buried shrimp by use of the hyponome excurrent in our current study in the foraging tank. If nautiluses are strict scavengers,
then the dual adaptation of the hyponome for movement and excavating may provide a greater probability of finding food.

In the field and in the laboratory, when any of the tentacles (medial or lateral) touched the bait, the other tentacles all directed toward the area that the first tentacle touched. At this point, although hidden from the camera in all observations but one in the field, the nautiluses exposed their beak and began tearing away at the prey item. As they were doing this, they also began to jet back and forth aggressively to pull at the bait. Perhaps this too serves dual purposes of assisting the nautilus in tearing away pieces of the prey and also jetting away quickly to finish consuming the prey elsewhere to reduce predation. However, this behavior was not exhibited by all nautiluses that found the bait and began to consume it. In the laboratory, once the shrimp was located or excavated, the nautilus rapidly jetted away, consistent with the idea that animals move quickly to safe locations after finding a prey item.

Lastly, the field observations support the hypothesis that nautiluses may be strict scavengers on decaying prey items. In many instances, nautiluses fed on the bait source even though they were close to, or covered by, living prey items, such as shrimp and hermit crabs. In 100% of the footage, the nautiluses showed no attraction or preference for the live prey items. Rather, the nautiluses fed on the bait source alongside the shrimp, hermit crabs, and other organisms. Several times, we observed shrimp crawling all over the feeding nautiluses (Figure 3.8) and then ‘riding’ on the hood and shell of nautiluses that jetted away (Figure 3.9). In these cases, it would seem that the nautiluses had ample opportunity to capture the shrimp, but again, the shrimp were left alone. Future studies investigating the diet preferences of nautiluses should provide concrete evidence of nautiluses naturally eat in the wild. Laboratory tests could enhance field study by giving animals choice tests between live and dead prey under similar conditions to
those described in my experiments. Because of the similarity of the behaviors of the 13 nautiluses approaching the bait source, hypotheses and predictions can be made about how they scavenge in the wild – to later be tested in the laboratory under naturalistic conditions.

Although nautiluses are able to learn and remember spatial and olfactory cues in laboratory trials (Crook and Basil, 2008; Crook et al., 2009; Crook and Basil, 2013), the lack of an increase in speed to find the shrimp during training and testing suggests that learning is not necessary for foraging and scavenging behaviors, supporting the notion that this is a strongly wired innate behavior (FAP) in response to odor, an adaptation that would suit an animal that is primarily an opportunistic feeder in an unpredictable environment.

It may be that the food we chose in the laboratory study was not a preferred food item for nautiluses, explaining the lack of success in locating the buried shrimp (though not reducing their search behaviors interestingly). While husbandry conditions for nautiluses have been significantly improved upon (Carlson, 1987; Barord and Basil, 2014), the diet of captive (and wild) nautiluses is still not well understood. The captive diet varies significantly at different institutions and public aquariums, for instance with variation in feeding rates of one large shrimp per day, seven days a week, to one shrimp per week (Pers. Obs). Yet all of these institutions maintain healthy populations, regardless of this variation. Understanding the exact “hunger level” of a nautilus is therefore extremely difficult. Thus, individual differences in appetite or preferred food item may have been responsible for the inconsistent success rate of the nautiluses actually capturing the prey. Perhaps the nautiluses had no motivation to consume the prey item because they were simply not hungry. All of the nautiluses, though, exhibited the characteristic foraging and scavenging behaviors during all of the trials when given the olfactory cue, again underscoring it is an innate behavior whether they are hungry or not.
The nautiluses performed similar behaviors under laboratory conditions as recorded via the BRUVS observations in situ. Both our BRUVs and foraging tests produced olfactory behavioral responses similar to previous studies (Bidder, 1962; Haven, 1974; Basil, 2000; Ruth, 2002; Basil, 2005) and also provided information on how nautiluses may search for prey with no visual cues of the actual prey item. The nautiluses performed distinct behaviors to search, locate, and capture prey (Figure 3.12). From these snap shots, we can determine a general model used to describe scavenging in nautiluses, most notably now including the ‘digging’ step (Figure 3.14).

Figure 3.12 Feeding stages of *Nautilus pompilius*; pre-scent (a), post-scent with preocular (proc) and post-ocular (pooc) visible and digital tentacles (dt) being extended (b), tentacles in search position with digital tentacles extended and digital lateral tentacles (dlt) drooping (c), and digging with tentacles and hyponome (h) to capture prey item (d). Tentacle groups labeled as in Ruth *et al.* (2002) and Basil *et al.* (2005).
In a resting, or unexcited state, nautiluses are retracted into their shell with the hood closing on the aperture of the shell, thereby protecting its tentacles and soft parts (Figure 3.14a). After the addition of an olfactory cue, the nautilus extends its tentacles slowly, the hood opens up, and the pre-ocular and post-ocular tentacles are visible (Figure 3.14b). If the olfactory cue is sufficiently strong and reliable as a potential prey item (or possibly mate in reproductive behaviors; Basil et al. 2002), the nautilus extends its digital tentacles and digital lateral tentacles in a wide posture referred to as the cone of search, or ‘cat whiskers’ (Figure 3.14c). This provides the nautilus with the greatest probability of locating prey in a dark environment where they may need to grope and feel along a coral reef face or muddy substrate to locate the prey. Finally, described here for the first time is the ability of nautiluses to dig for buried prey items (Figure 3.14d). During this process, it appears that all tentacles are involved in the digging. The hyponome also appears to be used in the process by blowing substrate out of the way so the tentacles can feel along the holes they dig. However, this may also relate to their status in the wild -- as they dig for prey items in the substrate they may be exposed to toxins that have that settle on and within the sediment. However, the rate and impact of processes such as sedimentation in nautilus habitat are unknown. Thus, future studies using core samplers would provide data on the impact that land based activities are having on nautilus populations.

Although nautiluses have long been assumed to be scavengers as well as active predators (i.e., Saunders, 1985), the combination of field and laboratory trials here provides the first substantiated evidence that nautiluses are indeed scavengers and may actually be strict scavengers, only consuming dead prey items. While the interactions between nautiluses and potential live prey items recorded by the BRUVS provide strong evidence that nautiluses do not feed on live prey items, additional laboratory tests with live and dead prey items would allow us
to directly test this hypothesis. From the laboratory foraging tests, we were able to observe at proximity the exact digging behaviors not recorded in the wild.

Using a multi-procedural approach to address questions about natural ecology and behaviors, especially in animals that live in extreme environments, like nautiluses, clearly works. By continuing to address questions of the natural history of these deep-sea, solitary animals using an integrative approach, we can answer critical questions regarding the natural ecology of nautiluses to support conservation efforts.
Chapter 4. Comparative Population Assessments of *Nautilus* sp. in the Philippines, Australia, Fiji, and American Samoa Using Baited Remote Underwater Video Systems

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4.1 Introduction

Nautiluses are part of an ancient nautiloid lineage that has existed for nearly 500 million year (Kroger et al., 2011). All living nautiluses inhabit deep coral reef slopes throughout the Indo-Pacific and comprise two genera: *Nautilus* and *Allonautilus* (Saunders and Ward, 2010; Ward, 1987; Ward and Saunders, 1997). Their habitat is constrained by depth implosion limits of 800 m (Ward et al., 1980), surface temperature limits of 25 °C (Carlson, 1987) and a nektobenthic life style (Ward and Martin, 1980), living in close association with reef slopes and ocean floors rather than in the mid-water or surface waters. These limitations effectively isolate local populations of nautiluses and may restrict gene flow, producing genetically distinct populations (Woodruff et al., 2010; Wray et al., 1995; Sinclair et al., 2011). This also limits the possibility of re-colonization events if local populations become depleted. Recent genetic work suggests the possibility that nautiluses have been undergoing a rapid adaptive radiation since the
Pliocene, and as such, there may be tens (or more) of currently unrecognized but separate sibling species unique to islands and land masses separated by water depths greater than the 800 m deep implosion depth (Bonacum et al., 2011). Thus, our best understanding of the genetic makeup of the various species is that the loss of any population results in an overall loss of genetic biodiversity.

Nautiluses can be captured using baited traps, which they can locate using chemoreception from significant distances. The ease of their capture using these traps coupled with the value of their shells, in both the shell and jewelry trade, have led to small and large scale fisheries in the Philippines, New Caledonia, and perhaps Indonesia in the 1970s onward (Haven, 1977; Alcala and Russ, 2002; Dekl Norte-Campos, 2005). However, lack of monitoring has obscured any objective understanding of either the size or biological effects of these fisheries on the standing populations in fished locales.

Nautiluses have a reproductive strategy typical of many deep water animals in showing slow growth to maturity (in this case, 12–15 years (Landman et al., 2010; Saunders, 1983; Saunders, 1984), low fecundity (0–10 eggs; Okubo et al., 1995; Uchiyama and Tanabe, 1996), and long developmental times (1 year Okubo et al., 1995; Uchiyama and Tanabe, 1996). As in the many fore reef slope fisheries of fish and invertebrates with similar life history strategies, such as the deep-water fish *Etelis*, Orange Roughy, and various deeper water, larger crabs such as *Geryon*, all of which have experienced documented population declines in specific localities where they have been heavily fished. These characteristics suggest that nautilus populations are inherently vulnerable to exploitation and may exponentially compound the effects of fisheries in reducing new recruitment. Yet to date, there have been only anecdotal reports describing population declines in two traditional nautilus fishing grounds (both in the Philippine Islands:}
Bohol Sea and Tanon Strait). While our own field observations have confirmed that nautilus fishing continues in the Bohol Sea, the Tanon Strait population may have already crashed by 1987 (Alcala and Russ, 2002; Ward, 1988). Yet, new evidence indicates that large numbers of nautiluses continue to be killed for their shells based on the first ever report of the nautilus shell trade by the United States Fish and Wildlife Service (DeAngelis, 2012). From 2006–2010, the number of nautilus shells or shell products (such as jewelry) imported into the United States exceeded 500,000 items. As a large number of these items were individual shells, these numbers attest to the effectiveness and scope of the global nautilus fishery.

The “normal” population density of distinct populations of either *Nautilus* (with four currently accepted species: *N. pompilius, N. stenomphalus, N. macromphalus* and *N. belauensis*) or *Allonautilus* (with two: *A. scrobiculatus* and *A. perforatus*) remained unknown until 2011 (Dunstan et al., 2011). Based on the large catches per trap from virtually all known *Nautilus* and *Allonautilus* trapping efforts, where as many as 60 nautiluses can be recovered from a single, 1 m$^3$ trap deployed for a single night (Carlson, 1987), it has been assumed that nautiluses are relatively common. However, new information has demonstrated that they are superbly adapted for discovering food from great geographic distances (Basil et al., 2000), leading to the alternative possibility that the large catches are misleading with regard to actual population numbers. With only one current estimate of a nautilus population available (Dunstan et al., 2011) and a survey-based study suggesting up to 80% declines in catch per unit effort in locations across the Philippines (Dunstan et al., 2010), it was imperative to assess additional populations of nautiluses.

Baited remote underwater video systems (BRUVS) are a relatively new method of population assessment in marine environments (Priede and Merrett, 1996; Cappo et al., 2007;
Harvey at al., 2012; Langlois et al., 2012). The majority of these studies were designed for use in relatively shallow coral reef systems. The use of BRUVS in the deep sea, however, has not been consistently researched. Although BRUVS have their own inherent biases, specifically related to the use of bait (Dorman et al., 2012), the use of BRUVS as a preferred alternative over other methods because it is less destructive (Harvey et al., 2007) and can provide estimates of relative abundance of economically important species (Lowry et al., 2007). When assessing unknown populations that are assumed to be under threat, such as nautiluses, BRUVS are also non-destructive and do not remove individuals from the population (Gladstone et al., 2012). Here, we used BRUVS to collect data from four previously un-sampled populations of nautiluses and provide the first quantification of the effect of fisheries on nautilus populations.

4.2 Materials and Methods

4.2.1 Ethics Statement

This study did not involve endangered or protected species and no animals were collected. Research in the Philippines conducted in collaboration with University of San Carlos and no permit required as no animals were collected. Research in Australia conducted under permit from the Great Barrier Reef Marine Park Authority and the University of Queensland Animal Ethics Committee. Research in Fiji conducted under permit from the Department of Fisheries. Research in American Samoa conducted under permit from the Department of Marine and Wildlife Resources.

4.2.2 Location

The study took place across four geographic locations in the Indo-Pacific with known nautilus populations. One fished population in the Philippines was compared to three non-fished
populations in Australia, Fiji, and American Samoa. The fished population in the Philippines was located in the Bohol Sea off the coast of Panglao, Philippines (9°35′ 18.87″ N, 123°43′ 44.54″ E). The three non-fished populations were along a transect of the Great Barrier Reef from Cairns to Lizard Island (16°37′ 28.91″ S, 145°53′ 07.35″ E), Beqa Harbour near Pacific Harbour, Fiji (18°19′ 40.24″ S, 178°06′ 30.86″ E), and Taena Bank near the harbor of Pago Pago, American Samoa (14°19′ 19.57″ S, 170°38′ 57.78″ W).

4.2.3 Data Collection

At each location, baited remote underwater video systems (BRUVS) were deployed to record the number of nautiluses attracted to bait over time. Each BRUVS unit consisted of a steel frame (100 cm×60 cm×75 cm), a Sony HD video camera in an underwater housing (Raytech Services PTY LTD), a white LED light source in an underwater housing, and a bait stick extending beyond the frame in view of the camera. While chicken meat was the primary bait used throughout the project (exclusively in the Philippines and Australia), limited resources in Fiji and American Samoa required the use of additional bait sources of tuna and mackerel when chicken was not available. Each BRUVS recorded up to 12 hours of video footage. The BRUVS were deployed at dusk (~1800 hours) and were retrieved the following morning (~0600 hours). The average deployment (soak) time was 12 hours. A total of three BRUVS systems were deployed at each site in Australia and spaced out 1 km from each other; the three BRUVS were not considered independent replicates for our analyses and were combined as one sample. The BRUVS were deployed in the Philippines, Fiji, and American Samoa using similar methods as Australia, the primary difference being that a total of two BRUVS were used instead of three for each night. Before deploying the BRUVS, a depth sounder was used to determine deployment depth. Although average depth between sites was ~350 m, deployment depths ranged between
300 and 400 m depending upon the location and topography of the ocean floor. A GPS unit was used to record the position of each deployment as well as the retrieval.

The BRUVS were deployed at each site multiple times across several days. The number of BRUVS deployments at each location varied due to adverse weather conditions and changing resources in the field. A total of four successful deployment days was achieved in the Philippines and American Samoa; three successful deployment days were achieved in Fiji; and a total of six successful deployment days were achieved in Australia across three sites; two deployment days at each site (site 1: 15°59'52.80"S, 145°51'15.66"E; site 2: 15°30'38.82"S, 145°49'2.40"E; site 3: 15°50'36.99"S, 145°48'45.42"E). As the Australian population inhabited a barrier reef, sampling multiple locations along the reef provided the most appropriate data. These data were then combined together to determine an average value for each population measurement, at each site.

4.2.4 Data Analysis

Each of the videos was reviewed and individual nautiluses were identified by their unique color pattern using the species recognition program, Hotspotter (Crall et al., 2013). The population density of each sampled area was calculated using footage from the BRUVS units. The total number of nautiluses was recorded from each of the videos. Next, the speed of the nautiluses in the video was recorded using a known frame of reference. The speeds were averaged at the sites to determine an average speed of 0.10 m s\(^{-1}\) (or 360 km hr\(^{-1}\)). These calculations are within range of several other swimming speed calculations in the literature (Dunstan et al., 2011; Ward et al., 1977; O’Dor et al., 1993). This average speed was multiplied by the total length of the video to determine the maximum distance the nautilus could travel to reach the recording site. This maximum distance value was then inserted into a formula (area of
a circle) as the radius to calculate the possible sampling area. Finally, the total number of nautiluses was divided by the sampling area to determine the population abundance of the area sampled. Statistical analysis was computed in R (R version 2.14.2). Means of populations and number collected by hours of observation were compared against each other using a paired T-Test. Secondly; the data was analyzed using a general linear regression model with ANOVA.

4.3 Results and Discussion

We used baited remote underwater video systems (BRUVS) in 2011–2013 to make quantitative measurements of the population abundance of nautiluses attracted to this system at four geographic locations in the Indo-Pacific: the Panglao region of the Bohol Sea, Philippines; the Great Barrier Reef along a transect from Cairns to Lizard Island; the Beqa Passage in Viti Levu, Fiji; and Taena Bank near Pago Pago harbor, American Samoa. From the video footage (see Video S1, S2, S3, and S4) we identified individual nautiluses using photographic identification of each specimen (Figure 4.1) through image recognition software (Crall et al., 2013) as the individual color patterns of nautiluses are unique. From these data we have calculated population abundance data at each geographic location (Table 4.1).
Even with our new observations from additional targeted observation sites, the largest number of nautiluses observed was measured from Osprey Reef and the Great Barrier Reef locations in Australia (93 total/2.01 per km$^{-1}$). Lesser numbers came from Beqa Passage, Fiji (20 total/1.58 per km$^{-1}$), followed by Taena Bank, American Samoa (22/1.48 per km$^{-1}$). The lowest numbers of all (6/0.25 per km$^{-1}$), by far, were measured at the Panglao locality in the Bohol Sea, Philippines. Comparison between sites using paired t-test and linear regression demonstrate a highly significant ($f = 9.99$; df = 44; P<0.001) difference between the Philippines site and the other four non-fished sites (vs. Australia $t = 22.2$; Fiji $t = 7.42$; A. Samoa $t = 11.18$; all P<0.001). Likewise, the attraction rates measured were greatest in the two Australian populations and lowest in the Philippines population, which was again significantly different than each of the non-fished sites (P<0.001).
Table 4.1 Population abundance values of the each location sampled including prior data from Osprey Reef, Australia\(^{10}\) representing all currently sampled *Nautilus* populations. doi:10.1371/journal.pone.0100799.t001

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of Nautiluses</th>
<th><em>Nautilus</em> Attraction Rate (N/hr)</th>
<th>Population Abundance (N/km²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osprey Reef, Australia</td>
<td>68</td>
<td>4.03</td>
<td>13.60</td>
</tr>
<tr>
<td>Great Barrier Reef, Australia</td>
<td>92</td>
<td>0.60</td>
<td>0.34</td>
</tr>
<tr>
<td>Beqa Passage, Fiji</td>
<td>20</td>
<td>0.79</td>
<td>0.21</td>
</tr>
<tr>
<td>Taena Bank, American Samoa</td>
<td>22</td>
<td>0.51</td>
<td>0.16</td>
</tr>
<tr>
<td>Bohol Sea, Philippines</td>
<td>6</td>
<td>0.09*</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

Next, we used the data from above (number of nautiluses and attraction rates) to calculate a population abundance at each location. The population abundance values mirrored the total number of nautiluses and attraction rates measured at each site with the Philippines site being significantly different than the non-fished sites (P<0.001), while the non-fished sites were not significantly different from each other. We also believe that the population measures reported here might, in fact, be overestimates at each site, given the ability of nautiluses to locate food across long distances as well as their confined (depth dictated) habitats. Thus, natural populations may be more dispersed and representative of lower levels of abundances and densities.

The use of BRUVS as an estimator certainly provides new information useful in evaluating the possible effects of fishing or other environmental change. Yet it is very clear that many variables are at play in determining the number of nautiluses attracted to the baited traps, with the rate, directionality, and other factors affecting the concentration of chemical scent moving out into the surrounded waters virtually impossible to quantify (Dorman et al., 2012; Harvey et al., 2007). Thus, the use of BRUVS alone has its limitations. On the other hand, the results obtained here are consistent with a conclusion that the fished, Philippines populations are significantly different in not only the numbers of nautiluses attracted to the bait each night, but
also in terms of the age-class structure of the attracted animals. For example the number of fully mature animals seen at Osprey Reef exceeded 80% (Dunstan et al., 2011), and this number is consistent with other studies of age class structure of sampled nautilus populations from Palau in the 1980s (Saunders and Spinosa, 1978; Saunders, 1983). Our work showed that less than 50% of the observed animals in the Philippines are mature.

While the differences in population abundance observed here might be artifacts of the methodology, or, if real, related to factors other than fishing (DeAngelis, 2012) (such as habitat change from increasing bottom temperatures, decreasing oxygen values, reduced food sources, and increased sedimentation), the presence of human fishing remains the most parsimonious explanation for smaller number of observed nautiluses in the Bohol region, and is the best explanation for what appears to be a complete abandonment of the once vigorous Tanon Strait nautilus fishery (the latter being geographically separated from the Bohol population). The fact that the latter population has not subsequently rebounded to a point where fishing has begun anew is certainly a red flag about that ability of nautilus populations to rebound even if all fishing were banned.

4.4 Conclusions

The greatest surprise of our data was the uniformly low population sizes among nautilus populations attracted to BRUVS at both the fished and non-fished sites. These low numbers suggest that extant nautilid species are vulnerable to unregulated (or perhaps even regulated) fisheries and may also be affected by other environmental changes in the deep sea marine ecosystem, of which even less is known than nautilus populations. It may be that factors other than direct nautilus fishing are, or soon will affect not just nautiluses, but other species of the still
poorly-known but large fore-reef slope communities and their environments of the tropical Indo-Pacific. Irrespective of this, these data provide valuable baseline information for future studies assessing fishery and/or environmental changes related to nautiluses and the flora and fauna of these deep sea habitats. We believe we have addressed significant gaps that have previously hindered regulatory and conservation agencies (DeAngelis, 2012) and the results reported here appear to validate older historical claims of nautilus population collapse due to the global nautilus shell trade, and argue strongly for immediate international regulation of the *Nautilus* and *Allonautilus* shell trade.
Chapter 5. General Discussion

The chambered nautilus is an iconic animal that is the inspiration and theme for many things in our world. From architecture to art, and even the novelty of collecting their shells, nautiluses are all around us. While we recognize the pattern and shape of the shell in a museum or logo, many people do not realize that an animal was once in that shell; let alone what it looked like when it was alive. The lack of a connection of this ‘beautiful’ shell has, in part, led to the absence of any management or protective strategies in nautilus fisheries. To strengthen our arguments for protecting this ancient animal, we must answer basic questions concerning their life history and ecology. Here, we address 3 critical questions related to their ecology: (1) how does Nautilus use information in its environment to make homing/movement choices, 2) how does Nautilus detect, track, and locate food, and (3) what is the current population size of nautiluses?

Nautiluses perform daily vertical and horizontal movements (Saunders and Spinosa, 1979; Ward and Martin, 1980; Carlson et al., 1984; Ward et al., 1984; Saunders and Ward, 1987; O’Dor et al. 1993; Dunstan et al., 2011) as evidenced by tracking studies using ultrasonic radio transmitters. Until the work of Dunstan et al. (2011), nautilus migration was considered to be the same for all nautilus populations: ascend at night to feed and descend during the day to avoid predators. Dunstan et al. (2011) recorded more variable movements throughout the entire 24 hour cycle and also long horizontal migration patterns at Osprey Reef.

Thus we know that nautiluses are able to perform these complex migrations. But what cues are they relying on to navigate their environment? We also know that nautiluses are positively phototactic (Muntz, 1987), which could be a possible cue, and have the ability to learn and remember landmark features using vision (Crook and Basil, 2009; Basil et al., 2012; Crook
and Basil, 2013). Here we tested two possible navigational tactics nautiluses may use, alone or in concert, (route memory or beacon homing) to find a goal (deep water) in a maze. Surely in the field both sources of information are available to them in some form (the silhouette of the reef, their own movements along the reef), however studying if they can use these cues requires controlled experiments in the laboratory.

Previous studies indicated that perhaps nautiluses were not good at remembering their route to a goal. However, here when we removed the beacon (visual cue), the nautiluses were still able to find the goal, presumably using route or directional memory. For an animal living in a three dimensional environment, partially in dark conditions, remembering the route to a location could be critical to survival. Changes in the environment affect how nautiluses weigh visual cues. When the beacon was shifted after learning, nautiluses performed less well in locating the goal, often because the salience of the beacon was so strong and they settled there first. However, when the beacon was shifted 90° the nautiluses appeared to detect the beacon had shifted too much relative to what they learned, and they ignored it and used route memory instead. So here we learned three aspects of nautilus homing and movements: 1) they can and do use route or directional memory, 2) there is overshadowing of route memory by the beacon, implying the two sources of information are learned at the same time but the beacon is more salient, and 3) there is a limit to the overshadowing – if the beacon location is significantly mismatched with what they learned (or even with their route memory), they ignore the beacon and rely on route or directional memory instead. Thus the memory streams are shared, but there is flexibility if landmarks become unreliable or are shifted. This begs the question, then, of what cues nautiluses rely on in the field.
Only now are we able to use consistent monitoring methods of nautilus habitat with underwater cameras (Dunstan et al., 2011; Barord et al, 2014). This footage shows habitat types ranging from rocky deep reef slopes to vast areas of sand with little to no rocks or similar visual landmarks. In at least one area of the Philippines the nautiluses migrated through both types of habitats within a 24 hour period. These migrations, then, may require the nautilus to switch between route memory and beacon homing, an ability that we have shown the nautiluses possess. The shared memory stream may also be beneficial during deep sea dust storms where vision is limited (as shown from video footage) so that nautiluses would be able to switch to route or directional memory in these cases.

While most cephalopods are characterized primarily as active predators, nautiluses have been described as scavengers and opportunistic predators (Saunders, 1984). We discovered here in our semi naturalistic foraging studies, and also by our observations in the field, that they are more likely strict scavengers. The seemingly modest question of what an organism eats is crucial to gaining a full understanding of its natural life history and ecology. Filling these knowledge gaps is critical to developing effective conservation and management plans to protect nautiluses from overexploitation.

Nautiluses are adapted for life in the deep sea where limited food items are available. Their strong olfactory sense and scavenging behaviors provide them with the highest possibility of locating food in the dark. However, their natural scavenging behaviors of trolling the substrate and digging for food may invariably do them harm. Agricultural runoff and sedimentation, among others, may impact life in the deep sea. As the nutrients and organic waste buildup, the possibility of toxins, such as metals, building up in the substrate increases. Bioaccumulation in nautiluses has already been documented (Bustamante et al., 2000; Pernice et al., 2007). Thus, as
nautiluses continue searching for food, they may also be consuming toxins and other chemicals
from land run off resulting from human induced causes. If nautilids, an lineage that has been
around for 500 million years, are affected by these changes, that will illuminate what is
happening in the entire scavenging community—a community that is critical to breaking down
detritus and decaying material so that it can be transferred back into the food chain.

Locating food in the wild most likely involves both navigation (route memory and/or
beacon homing) and also olfactory cues. There is no evidence to suggest that there are specific
sites that nautiluses travel to for food. What is more likely is that nautiluses rely on olfactory
cues to find food in their habitat. Given the dynamics of currents, it is probable that the cat’s
whiskers (cone of search) behavior is innate and stimulated by any type of decaying meat.
Nautiluses sense the cue in the currents, and depending on its strength/reliability of being a meal,
begin their fixed foraging and scavenging behaviors. During the search, the currents may
abruptly change or a stronger cue may be found and a new search begins. This, in part, may be
one of the reasons of the perceived sinusoidal patterns when searching for food. The nautiluses
are able to work towards pinpointing the location of prey while also scanning a wide area for
current cues and perhaps a surprise prey item in the mud found by dragging its digital lateral
tentacles in the mud. This pattern of a narrowing sinusoidal search as an animal approaches a
food source is also indicative of pure chemotaxis, also observed in Basil et al. (2000). This innate
search behavior affects their conservation and fisheries. In the case of fishing, the nautiluses find
their food but instead of decaying shark, it is raw chicken and the nautiluses are trapped. Their
acute olfactory senses make nautiluses easy to catch because many can be drawn in to a small
area and removed from the population. This disproportionate amount of nautiluses orienting to
the bait can quickly deplete a population.
We found that the population abundance of an area was significantly smaller in an area being fished (Philippines) than an area being unfished (Australia, Fiji, American Samoa). The baited remote underwater video systems (BRUVS) surveys provided a novel way to collect information on population abundance of nautiluses, as well as other aspects of their natural history. Our data strongly support that nautilus populations are in severe decline, since the creation of nautilus fisheries in the 1970s (Alcala and Russ, 2005; Dunstan et al., 2010; De Angelis, 2012). While this finding was expected, an unexpected finding was the overall small abundance levels of nautilus populations at each of the area sampled suggesting that all nautilus populations are susceptible to overfishing. The significantly smaller populations in the Philippines, a country that has already reported local extinction, provides strong support to approach governmental and conservation organizations to finally develop a management plan that benefits the communities and the nautiluses.
Chapter 6. Future Considerations

We cannot help nautilus populations without as thorough an understanding of their biology and ecology as possible. Because of the nature of their solitary lifestyle, deep-water habitat, and patchy distribution, field and laboratory studies working in concert is the most efficient way to address and test hypotheses about what is vital to their survival and in their daily lives. The future of healthy nautilus populations is dependent on effective management plans to ensure their survival. Effective management plans depend on the most reliable knowledge of the animal’s life history.

The most significant threat facing nautiluses is unregulated and mismanaged fisheries that supply a worldwide demand for the ornamental shell. However, this is not the only threat facing nautiluses. Similar to most other marine animals, nautiluses are also susceptible to other anthropogenic changes such as sedimentation, eutrophication, and climate change. The nautilid lineage has survived each of the five major extinction events. The lineage has survived climate change events and ocean acidification when many other species and lineages disappeared forever. Nautiluses are survivors and, given a chance, they will continue to survive and adapt. The only thing they need from us is to let them.
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