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Reconciling the Challenge of Aphanic Species Within Marine Conservation

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RECONCILING THE CHALLENGE OF APHANIC SPECIES WITHIN MARINE CONSERVATION

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Abstract

Aphanic species are those within a taxonomic complex that may not be readily distinguishable from other sympatric species. The existence of these species is becoming apparent at an increasing rate through the use of technological tools like molecular genetic analyses. A lack of clarity on the definitions of terms used to describe similar species, how these species are identified, and how prevalent they are can confound identification, description, and management of these organisms. This review collects and defines the terms used to describe these hidden species and suggests the use of the term aphanic for situations where additional information (and therefore classification) is not yet known. The review also addresses species identification methods and upholds the recommendation that newly proposed aphanic species should be validated by the use of two or more methods, such as morphological assessments alongside DNA identifications. Additionally, five historical case-study examples lead to the recommendation that management of newly-discovered aphanic species should remain managed under the species it is found within until information relating to each species' risk is understood. Information must then be pushed to and evaluated by the appropriate stakeholders to ensure effective management strategies.

Keywords: aphanic, cryptic, sibling, hidden, species, management, conservation, biodiversity, sustainability, marine, policy

Introduction

Overview

An accurate count of how many species currently exist is still heavily debated, and factoring in hidden (or aphanic) species brings additional complexity to the question. Aphanic species may have different vulnerabilities than the species they are hidden amongst. These varying biological characteristics can impact a known group or stock if the vulnerabilities are inadvertently exploited. This capstone review highlights the role of aphanic species within the much larger picture of biodiversity conservation. Additional impacts of aphanic species are explored regarding the management implications on environmental resiliency and marine environments.

Statement of Objectives

The objective of this capstone is three-fold. The first is to provide a literature review with a marine-centric focus on aphanic species. This includes a look at how species may be identified, an account of terms and descriptions used for aphanic species, and a comparative look at terms used within published works. The second objective is to review current marine management and conservation agencies and legal frameworks and to provide a historical account of management examples through case studies. The third objective is to suggest best practices for using the information on aphanic species to provide effective management guidance.

How Species are Identified

New species are being identified at an increasing rate, particularly in the area of aphanic species. This is largely due to improved technologies which illuminate distinctions between species which were difficult or impossible to identify in the past. There are multiple methods used to identify these differences, with some having evolved over time.

Morphology

In the times of the *Origin of Species* (Darwin, 1859), a species was a group of organisms that were identified by their morphological characteristics alone. In 1942, speciation was clarified by Earnest Mayr as groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups. This definition is commonly known as the biological species definition or the biological species concept. Given the difficulty of quickly or easily obtaining information on reproductive isolation, taxonomists have traditionally continued to utilize external morphology for identification (Teletchea, 2009; Jörger and Schrödl, 2013).

A visual distinction of species can be completed by assessing and comparing a multitude of structures and features, including but not limited to relative color, shape, size, and patterns exhibited. The color or relative length of a specific fin or the number or shape of vertebrae may be used as defining characteristics. Using morphology to identify a species can also result in identification ambiguities. Many species of fishes have diverse developmental stages, such as the various color morphologies seen with age and sex of scarid parrotfishes (Figure 1). Such identification ambiguity is also true for aphanic species, which are inherently difficult to separate by morphology alone (Figures 6, 7, 8, 9, & 10).

Figure 1. Princess parrotfish *Scarus taeniopterus* during juvenile and adult life stages. Image on left shows two juvenile *S. taeniopterus* and the right shows an adult *S. taeniopterus*. *Images courtesy of Study Blue (2017).*

There are additional complications that come from using morphometrics alone to identify individuals, even when characteristics do not change through life stages. First, the researcher making the identification must have knowledge to which identifying characteristics should be used. This is especially important for aphanic species and may require additional specimens for comparative purposes. When comparing two similar species, identification distinctions between two whole individuals can still be extremely difficult, creating the need for non-visual identification methods.

DNA and Molecular Approaches

In addition to morphological identifications, DNA and protein-based methods have historically been used to identify species. DNA-based methods in particular are being utilized at an increasing rate, resulting in an exponential increase in identified species (Bickford et al., 2007; McKenna, 2007; Jörger and Schrödl, 2013). Using these methods is important to researchers because unlike morphometric analyses, only a very small sample is needed to make a species determination and a whole organism may not need to be photographed or retained.

Of these methods, DNA based techniques have a number of advantages over those that are protein-based. Both techniques focus on the identification of polymorphisms that are unique to a particular species (Rasmussen and Morrissey, 2008). However, protein-based methods focus on examining relativities of peptide fragments which may not give clear results when analyzing closely-related species. This can be negated by developing an antibody for a specific protein (Woolfe and Primrose, 2004). Also, the biochemical properties of a protein testing material can be damaged by exposure to heat or by too much moisture loss (Mackie et al., 1999). Similarly, DNA can be compromised during processing but it is more thermostable than proteins. DNA also contains many non-coding regions, decreasing the potential loss of important identifying information (Lockley and Bardsley, 2000; Chapela et al., 2007). DNA is present in all cell types and is largely liberated of variances by tissue type or age, unlike proteins (Bossier, 1999; Civera, 2003).

DNA-barcoding works by screening reference genes to make it easier to assign individuals to a species or to help in the identification of new species (Hebert et al., 2003; Stoeckle, 2003). DNA tests use short, standardized regions of DNA base pairs to identify species under the assumption that interspecific variability should be greater than intraspecific variability which allows researchers to exploit differences (Hebert et al., 2003; Rasmussen and Morrissey, 2008; Kress & Erickson, 2012).

Of the several ways to utilize DNA for analysis, Polymerase Chain Reaction (PCR) is frequently used. This process amplifies DNA from a very small sample. PCR is often combined with other methods such as Restriction Fragment Length Polymorphism (RFLP), or Forensically Informative Nucleotide Sequencing (FINS) to aid in the identification of a species (Rasmussen and Morrissey, 2008). The methods used most frequently are PCR-RFLP, PCR-FINS and PCR-specific primers (Teletchea, 2009).

RFLP was the first method to be used to identify species via DNA (Manwell & Baker 1963; Ward, 2005). In this process, an amplified sample is exposed to enzymes which cut the DNA into fragments. The presence or absence of targeted enzyme recognition sites creates different fragment lengths of DNA, which are then separated by gel electrophoresis. Because different species or populations will have different recognition sites within the same stretch of amplified DNA, the varying bars will reveal alleles that can be used for Fixation Indent (F_{ST}). This is a measure of genetic differentiation and is among the most widely used measures for genetic differentiation (Willing et al., 2012).

PCR-FINS is the process of using single nucleotide polymorphisms (SNPs) to identify differences between species. With PCR-FINS, the purified PCR fragment's nucleotide sequence is identified and then compared to related sequences in a database. The sequence with the lowest genetic distance from the target fragment represents the group to which the species belongs (Bartlett and Davidson, 1992). SNPs are generally the most abundant sequence variants in genomes and are also used for F_{ST} to measure differences between populations (Willing et al., 2012).

PCR-specific primers can be used when previous knowledge of the desired material to be analyzed is available (Teletchea, 2009). When this species-specific information is available, SNPs may be identified or developed, and these primers will generate a fragment (run through gel electrophoresis) only in the presence of DNA from that particular species (Teletchea, 2009). Because SNP assays have historically been expensive to develop, studies using SNPs on non-reference organisms are rare (Narum et al., 2008), although new methods are being used to develop these assays and to significantly reduce costs (Tautz et al., 2010).

While many options for DNA-based species identification exist, DNA-based methods have been routinely criticized for multiple reasons (Moritz and Cicero, 2004; Will and Rubinoff, 2004; Meyer and Paulay, 2005). Researchers have claimed that some DNA-based species identification studies are not robust enough to make effective species determinations and single-gene barcoding should not be solely used as a basis for these determinations (Dupuis et al., 2012; Taylor and Harris, 2012; Adams et al., 2014). The primary foundations for these arguments include the basis that reference sets are not from one individual of that particular species, along with the consideration that DNA barcoding uses assumptions. The primary assumptions are that the reference set contains well-sampled intraspecific variation, reciprocally-monophyletic and hybrid introgression has not occurred, the most effective reference sequences were used, and the estimation of the gene evolutionary history is well-rounded. Given the multiple assumptions and uncertainties in relying on DNA-based species identification, it was argued by Ross et al. (2008) that unlike using identification methods based on morphology, molecular methods only allow us to make species identifications based on probabilities.

DNA-based techniques must always be optimized to provide undeniable and repeatable results to assist with making species identification (Woolfe and Primrose, 2004). This is especially important when reviewing aphanic species because species may have very similar DNA. It may be difficult to separate individual new species from individuals from the same species that simply express intraspecies variation (Rasmussen and Morrissey, 2008).

Behaviors, Geographic Ranges, and Habitats

In addition to species identification by the use of morphology or DNA sequence analysis, the use of other non-morphological data can assist with species determinations (Holyoake et al., 2001). A group of individuals exhibiting or lacking certain behavioral characteristics may be an indicator of a new species. Additionally, a species with a broad distribution may be a complex of aphanic species. (Quattro et al., 2013; Adams et al., 2014). Individuals that appears to be thriving outside of their normal species distribution may also indicate a new species and should be considered for evaluation.

Hidden Species Definitions

When it comes to the inclusion of aphanic species in literature, multiple terms have historically been used (Table 1). Cryptic species and sibling species were the first terms described in literature and were both coined by Ernst Mayr in 1942 following an extensive review of *The Origin of Species* (Darwin, 1859). These two terms dominate the existing literature. They were both originally given the same definition, but the sibling term suggests that the species in question shared a common, recent ancestor. Still, most researchers regard the terms cryptic and sibling species as synonymous (Bickford et al., 2006). These terms were followed by the term aphanic species, which was coined by George Steyskal to describe all cryptic or sibling species (Steyskal, 1972; Bickford et al., 2006). The term aphanic was derived from the Greek word aphanēs, which translates as unseen, hidden, unnoticed, inscrutable, or secret (Steyskal, 1972).

Table 1. Terms and definitions used to refer to species which are not readily identifiable

Term	**Definition (per first author)**	**Generally Accepted Definition**	**First Described**
Cryptic Species	Species that are so similar to one another that they are not recognizable as separate species, but they exist side by side without interbreeding.	Similar but genetically different species in which one expresses a cryptic coloration or pattern of the other.	1942 Ernst Mayr
Sibling Species	Species that are so similar to one another that they are not recognizable as separate species, but they exist side by side without interbreeding.	Similar but genetically different species that come from a common parent or stem from the same origin.	1942 Ernst Mayr
Aphanic Species	A group of species which satisfy the biological definition of species, but are not are not readily or reliably distinguishable morphologically.	A group of species which satisfy the biological definition of species, but are not are not readily or reliably distinguishable morphologically.	1972 George Steyskal
Pseudo-Sibling Species	Species that appear sibling but should not be named as such because of inadequacy of the morphological analysis.	Species that appear sibling, but can eventually be identified morphologically in certain life stages or when looking for the correct characteristics.	1993 Nancy Knowlton
Pseudo-Cryptic Species	Species that appear cryptic but should not be named as such because of inadequacy of the morphological analysis.	Species that appear cryptic, but can eventually be identified morphologically in certain life stages or when looking for the correct characteristics.	1993 Nancy Knowlton (term coined by extension of pseudo-sibling species definition)
Hyper-cryptic Species	Any taxon currently regarded as a single species or any related group of taxonomically confused species actually consists of 4-fold or more increase in new species.	A large group of independent species which coexist and are not distinguishable morphologically.	2014 Mark Adams et. al.

Although cryptic and sibling are often used interchangeably, cryptic is used more frequently in literature (Figure 2) and generally describes a species where it is not known if there is a common ancestor or when one species expresses a mimicry of another (Steyskal, 1972). A cryptic species named the Carolina hammerhead *Sphyrna gilberti* was described by Quattro et al. (2013) within the scalloped hammerhead *S. lewini* population of the western North Atlantic.

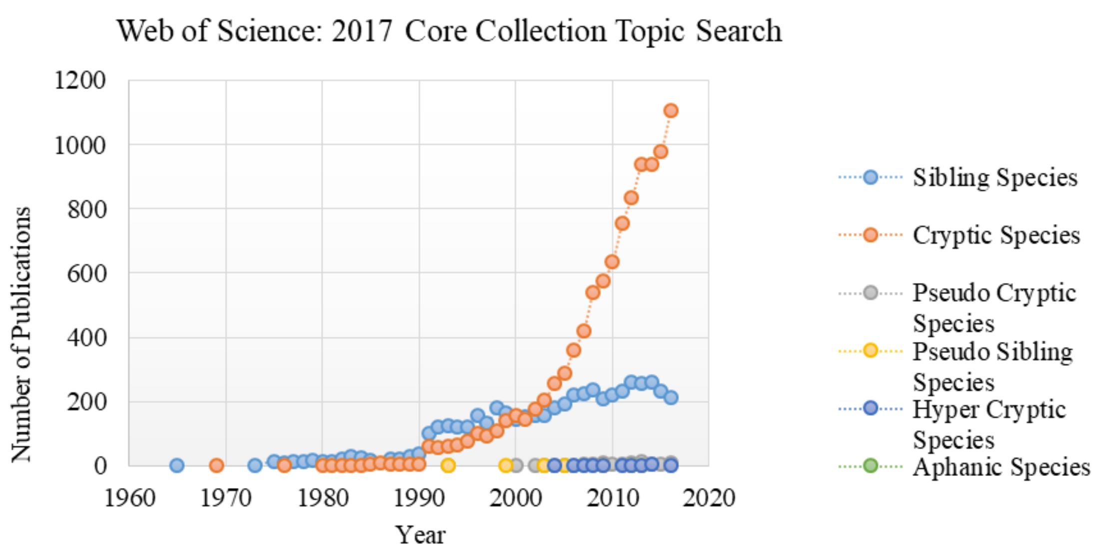

Figure 2. Annual Web of Science Articles Addressing Various Hidden Species

Sibling species describe morphologically similar but reproductively isolated populations and generally refer to two or more separate species with the assumption that they stem from the same origin (Mayr, 1943; Steyskal, 1972; Bickford et al., 2007; Adams et al., 2014). Sibling species were discovered within the yellow-eyed crab *Chiromantes obtusifrons* complex. *C. obtusifrons* was thought to have a widespread distribution from the West Pacific region to the eastern Indian Ocean; however, a study completed by Davie et al. (2013) discovered it actually included five sibling species, four of which were being described for the first time. A separate study confirmed the existence of two sibling species of marine gastropods *Columbella adansoni (*Menke, 1853) and *Columbella rustica* (Linnaeus, 1758) in which the larval stage distribution was virtually the only way to identify each species (Modica et al., 2017).

Additional terms describing hidden species have been noted in literature. Pseudo-sibling and pseudo-cryptic both refer to species that appear cryptic or sibling, but can eventually be identified morphologically within certain life stages or when looking for the correct characteristics. Under these definitions, most species defined as cryptic or sibling are actually pseudo-cryptic or pseudo-sibling and have simply been misnamed because the morphological analysis was deficient (Knowlton, 1993; Lajus et al., 2015). Hyper-cryptic species have been described as a species (complex) which is found to consist of a 4-fold increase or larger number newly-identified species (Adams et al., 2014).

Some aphanic species articles do not utilize any hidden species definitions when identifying the species. A 2006 study re-evaluated a species of billfish in Family *Istiophoridae* in need of validation, and genomic testing was able to confirm the existence of the roundscale spearfish *Tetrapturus georgii*, which has a strikingly similar morphology to both the longbill spearfish *Tetrapturus pfluegeri* and the white marlin *Kajikia albid*a (Shivji et al., 2006). This finding will be described in more detail as one of the capstone case studies.

Aphanic Species Prevalence

The question of how many species exist on our planet is difficult to answer and is complicated by the inclusion if aphanic species. Researchers currently rely on estimates which are based on underlying assumptions (Mora et al., 2011). While many biodiversity assessment studies acknowledge aphanic species (Bickford et al., 2007; Scheffers et al., 2012), most either do not explicitly address them or they do not regard them as significant contributors to biodiversity.

Aphanic species have be found throughout most organismal groups and biomes (Knowlton, 1993; Pfenninger and Schwenk, 2007; Pawlowski et al., 2012). Species which were originally described by morphological characteristics alone or for a species with a broad distribution may also have a higher chance of containing an aphanic species (Quattro et al., 2013; Adams et al., 2014). While this information may make identifying aphanic species simpler, it has been speculated that 91% of species in the ocean still await formal recognition (Mora et al., 2011). Increasing the number of studies that focus on aphanic species could lead us to a better estimate on biodiversity counts (Saez et al., 2005; McKenna, 2007).

Most researchers agree that there should be a smaller incidence of taxonomic outliers, especially under certain criteria. Species which are physically large (with easy to identify morphologic characteristics), common, well-studied, temperate region-dwelling, or easily accessible may be expected to have a smaller potential for containing an aphanic species (Adams et al., 2014) (Figure 3). While under these criteria the possibility of a species containing an aphanic species may seem small, it does not mean that are not present. Aphanic species such as these can be reviewed under Adams et al. (2014) in their study of mountain galaxias *Galaxias olidus*, Shivji et al. (2006) in their study of roundscale spearfish *Tetrapturus georgii*, and Quattro et al. (2013) with their study of Carolina hammerhead *Sphyrna gilberti*.

Group attribute	**Greater ⟵ Potential for cryptic biodiversity ⟶ Lesser**				
Kingdom	prokaryote/protists	⟶	'basal' eukaryotes	⟶	'derived' eukaryotes
Morphological complexity	simple	⟶	moderate	⟶	complex
Biome	tropical	⟶	temperate	⟶	extreme
Taxonomic effort	little	⟶	moderate	⟶	intensive
Molecular framework	none	⟶	basic	⟶	detailed
Ease of access to specimens	very difficult	⟶	not easy	⟶	easy
Infrastructure of host nation(s)	undeveloped	⟶	developing	⟶	developed

Figure 3. Summary of major factors influencing likelihood that a species or species complex may contain cryptic biodiversity. *Image from Adams et al. (2014).*

Importance of New Species

Biodiversity

There is mounting evidence that the stability of ecosystems is increased by biodiversity even in times of climate change and anthropogenic impacts (Tilman, 1995; Cleland, 2011; Loreau and de Mazancourt, 2013). Unfortunately, conflicting estimates of how many species exist highlight challenges when estimating environmental health and resiliency. Marine biodiversity loss in particular results in the ocean's impaired capacity to provide food, maintain water quality, and recover from perturbations. More specifically, Worm et al. (2006) found that loss of biodiversity resulted in an increase in the rates of marine resource collapses and a decrease in recovery potential, stability, and water quality. As expected, the ecosystem collapses were exponentially linked to diversity decline, and the restoration of biodiversity was able to increase productivity fourfold and decrease variability by 21%.

Anthropogenic effects on biodiversity should not be ignored. Humans have altered species diversity as the growth of the human population increases resource consumption and environmental pressures (Palumbi, 2001; Gascon et al., 2015). This has led to exploitation of and habitat loss for marine species. In the oceans, many invertebrate and fish species experience fishing pressure as a primary cause of mortality (Golletquer et al., 2014). Therefore, the idea that aphanic species are becoming extinct even before they are formally recognized is a valid possibility.

Other Considerations

Studying aphanic species may be important for reasons other than global biodiversity assessments. Recognizing new species is important for conservation planning, bio-prospecting, and biological control (Bickford et al., 2006). The accurate identification of individual species is necessary for things like evaluating possible pharmaceutical benefits, for the identification of invasive species, or to taxonomically identify components within foods or dietary supplements to ensure consumer safety and quality. DNA barcoding has been used to identify species of fish species in the marketplace by analyzing shark fin samples and tuna muscle tissue (Abercrombie, 2005; Vinas and Tudela, 2009).

A consideration is to note that funds set aside for environmental sustainability and management do not increase with the discovery of each new species. Therefore, the addition of new species to management lists may cause additional strain on those who manage our ocean's resources. The conservation status of each newly-discovered species should be assessed first (Murphy et al., 2013; Adams et al., 2014). Then management plan changes can be addressed if needed. If new management, regulations, or changes are necessary, the time and costs needed to implement them must be considered. Ultimately, conservation management should consider all species and their vulnerabilities.

Implications for Fish

Commercial fisheries production from wild stock harvests is estimated at >90 million tons annually, and fish product exports including aquaculture from the developing world are worth more than all other agricultural commodities combined. While the commercial value may seem like a large consideration, the value is increased when we consider the artisanal, recreational, cultural, and spiritual importance that fisheries may provide (Gascon et al., 2015). Therefore, species identification related to fish is of particular concern because of these economic effects. More than 8% of the animal protein consumed by humans annually comes from fish, with one in five people depending on fish as their primary protein source (United Nations, 2017).

If one stock is found to be composed of multiple species, the value given to that entire stock will change when applied directly to the smaller subset of aphanic species. Aphanic species identified within one perceived stock can result in large changes to the value of a targeted stock and the impacts can be exponential. The fishing mortality rate for the maximum sustainable yield of a population (fMSY) represents the target rate of fishing effort on a stock which would allow the stock to continue to thrive while effectively be fished at the same time (NOAA, 2017). If one stock is found to be a complex of two or more species, the fMSY that is being targeted is higher than each population’s fMSY, exposing both of the populations to overfishing (Figure 4).

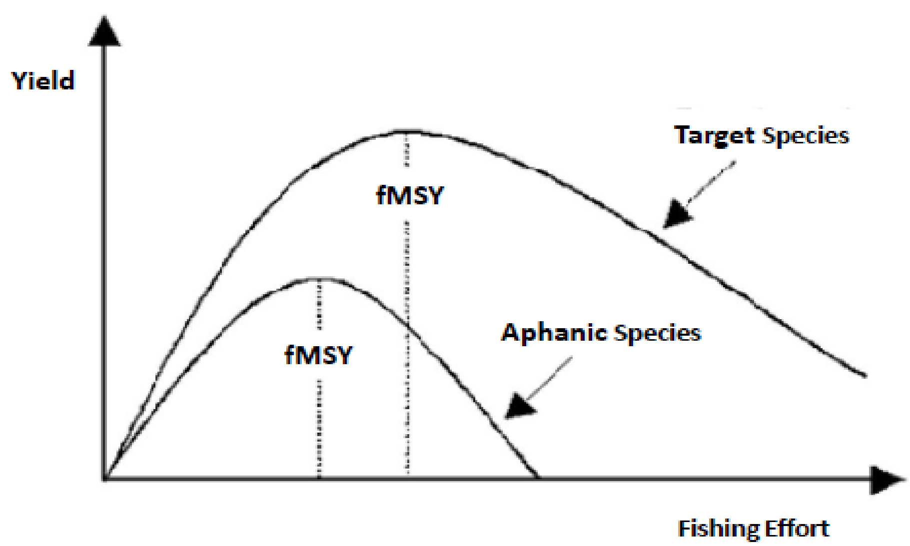

Figure 4. Relationship between fMSY of target species and aphanic species.

Management Authority and Objectives

As new aphanic species are being identified, the push for management or protections of these species has increased (Knowlton, 1993; Saez and Lozano, 2005). However, a general lack of aphanic species biological information can make conservation attempts more difficult. Different criteria may need to be evaluated in order to evaluate the risk to each species for management purposes. The ratios or stock sizes of each species involved in a newly-identified species complex or the current management of each species may need to be evaluated. If appropriate management is not in place, a stock can plummet quickly. This effect can also be intensified by illegal, unreported, and unregulated fishing, which has been increasing worldwide (United Nations, 2017).

Current Management Overview

While there is not a standard, formal process or repository used for identifying a marine species, there are many management divisions and statutes that are involved in management and conservation of species from various levels. First, United States Department of Commerce contains the National Oceanic and Atmospheric Administration (NOAA) which focuses on the conditions of the oceans and the atmosphere. This agency is responsible for establishing governing statutes for marine conservation purposes. A division of NOAA responsible for the conservation of living marine resources and their habitats is the National Marine Fisheries Service (NMFS) (NOAA, 2017).

NMFS primary authority comes from three Federal statutes: the Magnuson-Stevens Fishery Conservation and Management Act (MSA), the Endangered Species Act (ESA), and the Marine Mammal Protection Act (MMPA) (Table 2). The MSA is the primary law responsible for governing management of marine fishes. The ESA replaced the Endangered Species Conservation Act of 1969 and is responsible for endangered or threatened species conservation. The MMPA governs the taking of marine mammals so that the species is still sustainable or can recover to their optimum sustainable population size. It is important to note that the different objectives mandated by each law (MSA, ESA, MMPA) create the need for different definitions of conservation units (i.e., species) (NOAA, 2017) (Table 2).

Table 2. NOAA Report 2006, NOAA 2017
U.S. governing statutes and objectives related to conservation of living marine resources

U.S. Statute	Objective	Definition of Conservation Unit by federal statute
Magnuson-Stevens Fishery Conservation and Management Act (MSA)	The MSA governs the exploitation of fish stocks for the maximum net benefit of the Nation, while preventing overfishing and rebuilding overfished stocks of fish to biomass levels capable of producing Maximum Sustainable Yield (MSY).	Allows for management units that may contain multiple species as members of a complex, but the concept of demographically independent stocks within a species is commonly used to determine the status of fishery resources. demographic independence is an appropriate basis for identifying conservation units (distinguishing among populations or stocks
Endangered Species Act (ESA)	The ESA governs the taking of species that have an elevated risk of extinction to ensure that effects of human activity are restricted to levels that would allow recovery of the species to the point it is no longer threatened or endangered.	Should be substantially reproductively isolated from one another to be listed under this act. To be considered an Evolutionarily Significant Unit (ESU), it must represent an important component of the evolutionary legacy of the species.
Marine Mammal Protection Act (MMPA)	The MMPA governs the taking of marine mammals so that the total of such taking is sustainable, that is, it would allow populations of marine mammals to recover to or to be maintained within their Optimum Sustainable Populations (OSP).	Include keeping populations or stocks of animals above their Optimum Sustainable Populations OSP levels. demographic independence is an appropriate basis for identifying conservation units (distinguishing among populations or stocks)

Magnuson-Stevens Fishery Conservation and Management Act (MSA)

The MSA of 1976 remains the primary law governing marine fisheries management in U.S. federal waters. It fosters the long-term biological and economic sustainability of our nation's marine fisheries, with sustainability being defined by activity leading to the harvesting of fish. Since its enactment, two significant revisions have been made to the MSA by Congress; once in 1996 when the Sustainable Fisheries Act was established and again with the Magnuson-Stevens Fishery Conservation and Management Reauthorization Act of 2006. Prior to the MSA, waters beyond 12 nautical miles were considered international and were fished as such. The MSA extended regulatory authority from state waters out to 200 nautical miles offshore (NOAA, 2017).

The MSA established eight Regional Fishery Management Councils (Figure 5), each with a primary responsibility to develop fishery management plans (FMPs) within its jurisdiction. Each FMP is required to specify objective and measurable criteria for determining when a stock is overfished. The FMPs are required to comply with conservation and management requirements including the *10 National Standards* principles, which focus on sustainable management in fisheries. These 10 standards focus on optimum yield, scientific information, management units, allocations, efficiency, variations and contingencies, costs and benefits, communities, bycatch, and safety of life at sea (NOAA, 2017).

The MSA was revised by the Magnuson-Stevens Fishery Conservation and Management Reauthorization Act of 2006. This Act aimed to make some much needed changes. These changes included establishing catch limits and accountability measures, promoting management strategies based upon the market, and to add in programs for limited access privilege. It also included changes to better utilize peer review to improve the science-based roles within management and to address illegal, unregulated, and unreported fishing and bycatch, amongst other changes (NOAA, 2017).

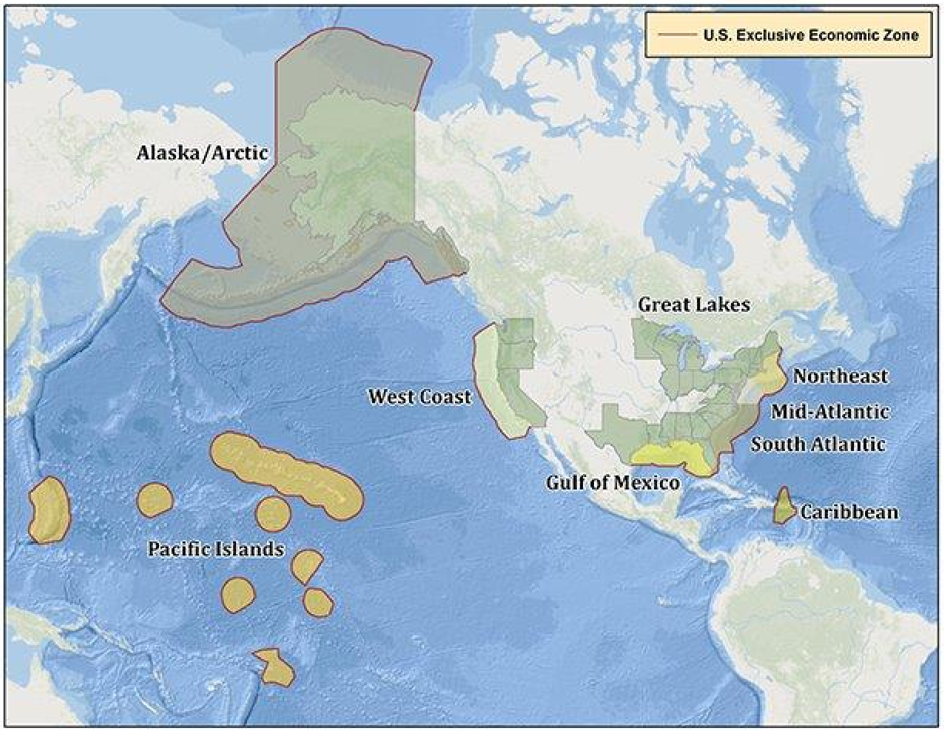

Figure 5. United States Regional Fisheries Management Councils: Management Areas. *Image courtesy of Image courtesy of Fisheries Councils (2017)*

Management of tuna, billfish, and swordfish fall under NOAA by authority of the MSA and the Atlantic Tunas Convention Act (ATCA). The ATCA was established in 1975 and was put in place to authorize NOAA to administer and enforce the International Commission for the Conservation of Atlantic Tunas (ICCAT) provisions. The ICCAT has 48 contracting international parties and conducts annual stock assessments of Highly Migratory Species (HMS) such as tunas, swordfish, and billfish across the Atlantic Ocean, Mediterranean Sea, Caribbean Sea, and the Gulf of Mexico. Currently, there are about 30 species of direct concern to ICCAT (ICCAT, 2017). The primary focus of ICCAT is to rebuild overfished stocks and to allow for sustainable fishing of these species. Nations within ICCAT negotiate management recommendations and catch quotas based upon stock assessments and enforce as appropriate. Enacted ICCAT recommendations, such as quotas, minimum sizes, and trade restrictions are binding to U.S. Atlantic fisheries and are subsequently implemented with domestic regulatory measures (NOAA, 2017).

Marine Mammal Protection Act (MMPA)

The MMPA was enacted in 1972 and protects all marine mammals. The primary purpose of the MMPA aims to prevent species or stocks from falling below their optimum sustainable population levels. Congress passed this act after some marine stocks were found to be at risk due to human activities. The MMPA prohibits the take of any marine mammal in U.S. waters and by U.S. citizens with few exceptions. Take is defined as actions to harass, hunt, capture, or kill or attempt to do so. This prohibition includes the import of marine mammals or products into the United States of America. A 1994 amendment to this Act was established to allow certain exceptions to the take prohibitions. These include but are not limited to any takes which are incidental to specified activities, when permits are authorized for scientific research, for stock assessment purposes, or for pinniped-fishery interaction studies (NOAA, 2017).

Endangered Species Act (ESA)

The U.S. Fish and Wildlife Service (FWS) and the National Marine Fisheries Service (NMFS) under NOAA share responsibility for implementing the ESA. The ESA was passed by Congress in 1973 with a primary purpose to conserve threatened and endangered species and their ecosystems by listing and overseeing management of these species. The ESA defines a species as endangered if there is a threat of extinction throughout all or a significant portion of its range, and they are listed as threatened if likely to become an endangered species within the foreseeable future. As of 2017, approximately 2,270 species are listed as endangered or threatened, 157 of which are marine species (NOAA, 2017).

There are two ways to list a species as endangered. NMFS can initiate a status review of a particular species, or petition can be initiated by a person or organization and proposed to NMFS. A species may be listed as endangered or threatened due to a number of factors, some of which include habitat destruction or overutilization, disease or predation, inadequacy of regulatory mechanisms, or other natural or anthropogenic factors. Decisions are made after a review of any available scientific and commercial data, any current conservation efforts, and the species’ current status (U.S. Fish and Wildlife Service, 2017).

Additional International Efforts

In addition to U.S. domestic and international laws and regulations, the International Union for Conservation of Nature (ICUN) is a non-governmental organization which is responsible for identifying Marine Protected Areas (MPAs) and for maintaining the IUCN Red List. Although non-governmental organizations like the IUCN are not strictly bound to U.S laws and regulations, their assessments and other products are generally regarded as unbiased and may influence multiple countries and their rulemaking processes.

The IUCN Red List was established in 1964 and uses a graded series to identify the threat level of a species (ICUN, 1994). The risk level of a species is based on five quantitive criteria: a population reduction, a small distribution and decline, a small population size and decline, a very small or restricted population size, or a quantitive population analysis. The threatened list contains three levels identified as vulnerable (20% reduction in 10 years or 3 generations), endangered (50% reduction in 10 years or 3 generations), or critically endangered (80% reduction in 10 years or 3 generations). Listing species as endangered makes it illegal to do or attempt anything including take, harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect the species, with similar prohibitions usually extend to threatened species (Musick, 1999; NOAA, 2017).

Most fish listed on the IUCN Red List are there because of population reductions (Musick, 1999). A reduction per the IUCN is defined as a decline in the number of mature individuals of a percentage specified over a specified time frame (IUCN Red List, 2017). This concept is especially important to aphanic species because the reduction may affect each species that make up the complex differently, which could severely compromise one of the species.

MPAs have proven to be effective tools for conserving marine ecosystems. They restrict human activities for conservation purposes, such as being named as no-take zones with sustainable activities buffer zones (Di Franco et al., 2016). MPAs are managed by local, state, territorial, native, regional, national, or international authorities and differ substantially among and between nations. MPAs are generally an area of the intertidal or subtidal terrain which have been reserved as to protect part or all of the enclosed environment. An MPA area includes the overlying water and associated flora, fauna, historical and cultural features (Kelleher, 1999).

Case Studies

Applying the previously discussed information to aphanic species as they have been identified provides real-world examples on applications. Five case studies will be described and management outcomes will be addressed, which will be summarized to provide a baseline for future recommendations.

Case Study 1: Roundscale Spearfish

Shivji et al. (2006) re-evaluated the roundscale spearfish *Tetrapturus georgii*, an aphanic species of billfish in Family Istiophoridae. This species was first described by Lowe (1840) based upon a catch location and cursory description of only one specimen. A subsequent study to validate the species was conducted by Robins (1974), but only utilized four specimens from the Mediterranean and northeastern Atlantic (Shivji et al., 2006). Due to the classification based upon such few number of individuals and the limited range of the associated distribution, validation of this species was still needed (Nakamura, 1985). The 2006 study provided both the genetic and morphometric analysis needed, however the 1974 study provided the extensive morphological description that is still relied upon today (Shivji et al., 2006).

Within this study, *T. georgii* specimens (n=16) were collected from western North Atlantic waters by way of fishery observers with the NMFS Pelagic Observer Program from 1996 to 2005. All specimens were described as resembling either *K. albidus* or *T. pfluegeri*, but looked slightly different based on scale morphology (Figure 6). From these specimens, photographs, measurements, and samples were collected and reviewed to evaluate scales. The samples were taken from the mid-lateral side of each animal a few centimeters anterior to the pectoral fin, and all standard measurements were taken along with the distance between the anal opening and the origin of the first anal fin. Measurements were also taken from the greatest height of the first anal fin. For comparative analysis, samples and measurements were evaluated for *K. albidus* (n=13) and *T. pfluegeri* (n=9) which had been landed in the same fishery (Shivji et al., 2006).

Figure 6. Morphological comparison of *Kajikia albidus* (top) and *Tetrapturus georgii* (bottom). *Image courtesy of Guy Harvey Research Institute (2010).*

Alongside morphometric analysis, genomic DNA was analyzed via PCR- specific primers. Approximately 25 mg of muscle tissue was analyzed and evaluated for different species to make comparisons (Table 3). These multiple billfish species were compared so that a perspective of evolutionary genome distances could be included in the results.

Table 3. Shivji et al., 2006
Species analyzed and compared via DNA

Species	**Specimens evaluated**
Roundscale spearfish *Tetrapturus georgii*	10
White marlin *Kajikia albidus*	4
Longbill spearfish *Tetrapturus pfluegeri*	4
Mediterranean spearfish *Tetrapturus belone*	4
Sailfish *Istiophorus platypterus*	4
Blue marlin *Makaira nigricans*	3
Black marlin *Makaira indica*	1
Striped marlin *Tetrapturus audax*	1

Results showed that there were small differences between species as described in the 1974 study, however these characteristics may be too subtle and therefore impractical to rely on for identification. Of these differences, the scales of *T. georgii* matched the description of scales reported for the four specimens by Robins (1974) and were notably soft and rounded at the anterior end with two to three posterior points. Scales of the *K. albidus* and *T. pfluegeri* were stiffer and pointed at the anterior end with posterior points of one to two for *K. albidus* and two to five for *T. pfluegeri*. Additionally, the ratio of the distance from the anus to the first anal fin origin to the maximum height of the first anal fin for *T. georgii* did not overlap with either ratio for *K. albidus* or *T. pfluegeri*. While these characteristics may make it easier to identify the species, they may be difficult to identify for untrained recreational or commercial fishers and when attempting to identify live fish in the water (Shivji et al., 2006).

A follow-up study by Bernard et al. (2013) described a much larger distribution of this species than previously expected. Morphology and DNA via PCR- specific primers were used to validate the species and the distribution was found to include much of the western South Atlantic and central North Atlantic, overlapping even more than previously thought with the sympatric species of *K. albidus* and *T. pfluegeri.*

Misidentifications of *T. georgii* as *K. albidus* have significant implications for management and conservation, especially as they relate to catch records which form the basis for stock assessments (Bernard et al., 2013). The updated distribution information further complicates this issue because these misidentifications now include a larger set of fish which were utilized in stock assessments.

Currently, all billfish species are prohibited for harvest for commercial purposes. *K. albidus* and *T. georgii* landings are permissible only when the vessel has a valid Highly Migratory Species (HMS) charter or angling permit or when the vessel is registered and participating in a registered HMS tournament (NOAA HMS Commercial Compliance Guide, 2016). When it comes to the actual conservation statuses of each species, *K. albidus* is listed as vulnerable on the IUCN Red List but is not listed as endangered or threated under the ESA, *T. pfluegeri* is listed under least concern on the IUCN Red List and is not listed under ESA protections, and *T. georgii* is listed as data deficient in the IUCN Red List and is not included under ESA protections (IUCN Red List, 2017; ECOS, 2017).

From a fisheries perspective, the roundscale spearfish is being managed under the *K. albidus* population, which allows for the take of fish with a 66 inch fork length or larger when appropriate permits and situations make the harvests permissible (NOAA HMS Commercial Compliance Guide, 2016). Managing the species as one increases the chances of conservations of both species because misidentifications can happen so easily. Focus should be directed towards the level of protections on *K. albidus*, as new information about the distribution of *T. georgii* should call into question previous *K. albidus* population assessments which served as the basis for the current HMS limits.

Case Study 2 – Carolina Hammerhead

A study by Quattro et al. (2013) described a new aphanic species subsequently named the Carolina hammerhead *Sphyrna gilberti*. This species is difficult to identify against the sympatric scalloped hammerhead species *Sphyrna lewini* (Figure 8). The primary purpose of this study was to evaluate if there was a new species of hammerheads and if so, which if any characteristic would separate the original species from the cryptic specters.

Within this study, 80 possible aphanic hammerheads within the *S. lewini* population were collected from the coastal waters of South Carolina from 2001 to 2003 using longline and gillnet gear. Species identification was analyzed by way of morphometric and DNA analysis. Tissue samples were removed from the pectoral fin for DNA processing by PCR- specific primers. Individuals were retained for measurements and evaluation of morphometric features both visually and by way of radiographs. For morphological data, methods were used to remove possible variability due to body size to improve the reliability of the data. Results from this study showed significant differences between species in the mean or median lengths for pre-pectoral length and inner narial groove length. Additionally, a significant difference was found in the number of precaudal vertebrae, with 92–99 occurring for *S. lewini* and 83–87 for *S. gilberti*. Of the 80 individuals tested, both DNA and morphometric analysis concluded that 54 individuals belonged to the new species, while the remaining were identified as *S. lewini* (Quattro et al., 2013).

Currently, NMFS has not yet made a distinction between the two species for management purposes, so the aphanic species is managed under the *S. lewini* population (Quattro et al., 2013). *S. lewini* are listed as endangered for eastern Atlantic and eastern Pacific regions and threatened for central/ southwest Atlantic and indo-west Pacific regions (NOAA HMS Commercial Compliance Guide, 2016) and are listed as endangered on the IUCN Red List (IUCN Red List, 2017). *S. lewini* (and by extension, *S. gilberti*) are considered an Authorized Species by NOAA HMS and regulations allow the harvest of one per vessel per trip at 78” fork length or larger. Restrictions do apply. These sharks may not be taken by vessels with pelagic longline gear onboard and vessels may not possess these sharks while in possession of tunas, swordfish, or billfishes (NOAA HMS Commercial Compliance Guide, 2016).

Current management of the aphanic *S. gilberti* within the *S. lewini* population may increase the chances of conservations of both species because misidentifications can happen so easily. Still, evaluation of the incidence of the aphanic species within the *S. lewini* population could affect future management decisions. This may create the need for a re-evaluation of the level of protections on *S. lewini*. Outcomes could then influence change to the HMS regulations on the species if needed.

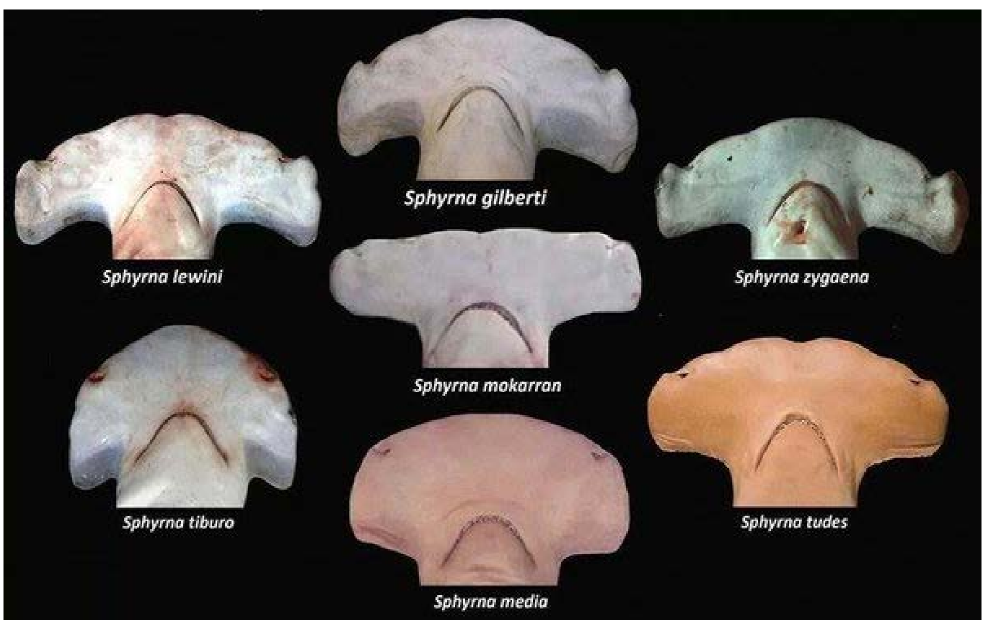

Figure 7. Hammerhead species morphology. *Image courtesy of Tiburones en Galicia (2014)*

Case Study 3- Mytilid Mussels

Blue mussels exist on both coasts of the North Atlantic (including the Mediterranean Sea) and the North Pacific and are commonly utilized for human consumption. Researchers discovered that blue mussels are actually an aphanic complex of three species. In this complex, the European blue mussel *Mytilus galloprovincialis* was identified as an invasive species within the native southern California habitat of *Mytilus trossulus* and *Mytilus edulis*. Since these species cannot be identified morphologically (Figure 8), the invasion happened without being noticed and DNA testing was necessary for speciation. Archeological samples were compared to present species to identify changes and determine if a decline of the native populations was consistent with the introduction of the invasive species (Geller, 1999).

In this study, dry tissues of mussels were collected from samples stored from 1884, 1871, and 1900 and they were compared to current samples. The mussels' DNA was processed by PCR- specific primers and tests concluded that *M. trossulus* was the sole species present in historical samples, indicating that a decline of the species happened after year 1900. As the population of mussels were present continuously in this region since 1900 (although an increase in population was noted in the 1940s) data suggests that *M. trossulus* were present during the initial invasion of *M. galloprovincialis* (Geller, 1999). It has been identified that these aphanic invasive mussels have shown more resilient characteristics than their counterparts such as a higher ability to survive in warmer and higher salinity environments when compared with the native mussel species (Tomanek and Zuzow, 2010). This highlights how easily an entire species can be affected while going virtually unnoticed.

While all three species in question are not listed under either the ESA or the IUCN Red List, the species have implications for human safety. Given that these mussels are regularly consumed, studies have been completed addressing contamination within these species (De Witte et al., 2014) and for environmental monitoring (Hamer et al., 2004; Payne et al., 2008). While all three species are all currency being managed and consumed as one species, further studies are needed on the contamination levels of each individual species within the same environment so that management can be modified to protect consumers, if needed.

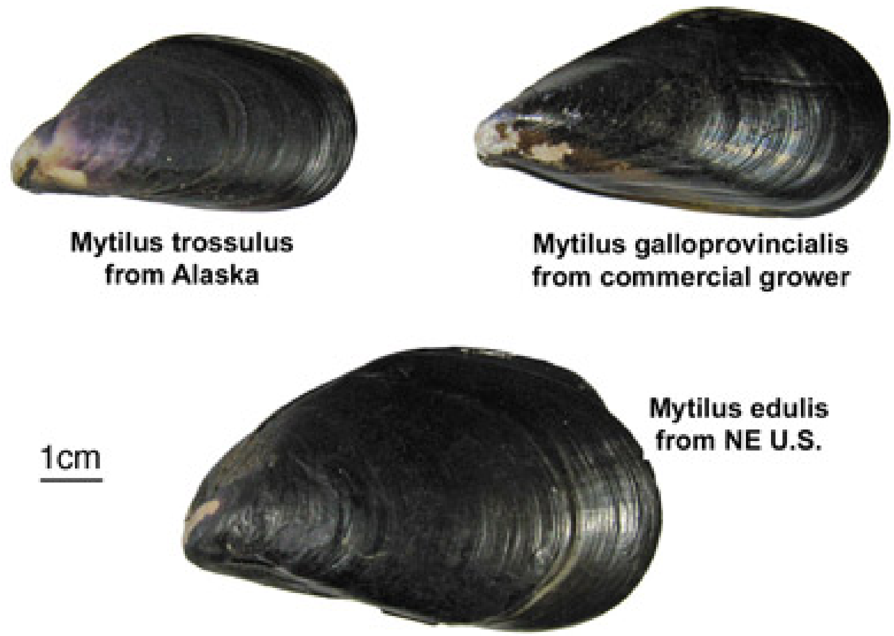

Figure 8. *Mytilus* Complex. *Image courtesy of A Snails Odyssey (2012)*

Case Study 4 – Bigeye and Yellowfin Tunas

There are eight species of tunas in the genus *Thunnus* and identification of these species have at times proven to be difficult. Identification is particularly difficult between the bigeye tuna *Thunnus obesus* and yellowfin tuna *T. albacares* (Vinnas and Tudela, 2009) (Figure 9). While field guides do exist to help fishermen identify these fishes, misidentification of these species still occur. If these misidentifications are then reported as accurate, catch data and population estimates can be affected.

Figure 9. Bigeye and Yellowfin Tunas
Image top: *T. albacares* top, *T. obesus* bottom; Image bottom: *T. obesus* top, *T. albacares* bottom. *Images courtesy of ISSF (2017)*

There are characteristics that can be used to identify *T. albacares* from *T. obesus* but they can be subtle and at times unreliable. Characteristics that are internal such as liver and swim bladder morphology or external such as head and eye morphology can be indicators for one species or the other. In addition to these, markings and colorations of bodies and fins can be used to make species determinations while individuals are fresh, however colors and markings may fade as time out of the water increases (Vinnas and Tudela, 2009). This complicates species identification between *T. albacares* and *T. obesus*.

The United States is involved in the management and conservation of tuna and tuna-like stocks, with NOAA negotiating international measures. Of these are the International Commission for the Conservation of Atlantic Tunas (ICCAT), the Inter-

American Tropical Tuna Commission (IATTC), and the Western and Central Pacific Fisheries Commission (WCPFC). In addition to ICCAT, the IATTC is responsible for the conservation and management of tuna and other marine resources in the eastern Pacific Ocean, and the WCPFC provides a forum for long-term conservation, sustainable use, management and of highly migratory fish stocks (tunas and billfishes) in the western and central Pacific Ocean (NOAA, 2017).

T. albacares and *T. obesus* are currently managed as two separate stocks and there are slightly different conservations regulations for each species. Both are considered authorized species with an HMS permit, with the recreational *T. albacares* retention limit at three per person and no retention limits on *T. obesus*. The minimum size for both *T. albacares* and *T. obesus* is 27 inches curved fork length (NOAA HMS Commercial Compliance Guide, 2016). *T. obesus* is listed as vulnerable on the IUCN Red List and *T. albacares* is listed as near threatened, while neither species has been listed under the ESA (IUCN Red List, 2017; ECOS, 2017). Because there are differences between NOAA and the IUCN defined threat levels to each species, it could be argued that the stocks should be reassessed for vulnerabilities and then both managed as more vulnerable of the two.

Case Study 5 – Pilot Whales

The long-finned pilot whale *Globicephala melas* and the short-finned pilot whale *G. macrorhynchus* are two large species of dolphin which are protected under the MMPA. It is very difficult to distinguish between the two species, especially at sea. While they do have slight differences in morphology (Figure 10), the primary means of identifying each species is by seasonal spatial distributions (NEFSC NOAA, 2014). This means of identification is still not completely reliable as the geographic range for these species may still overlap in some areas (NOAA, 2017).

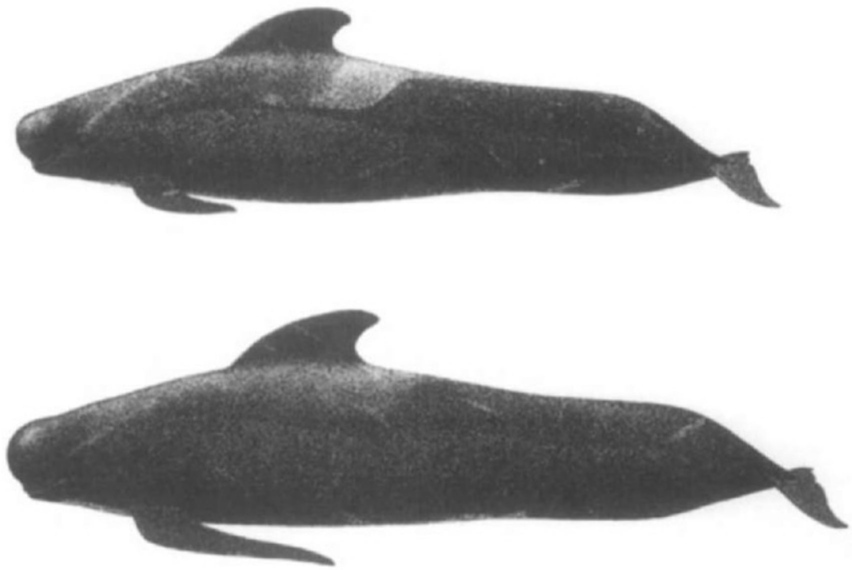

Figure 10. Long-finned and short-finned pilot whales. Full-body view of *G. macrorhynchus* (top) and *G. melas* (bottom). *Image courtesy of what-when-how (2017)*

Both *G. macrorhynchus* and *G. melas* are currently at risk. Most of this risk can be attributed to unintended interactions with fishing gear. Commercial fishing gear such as gillnets, longlines, and trawls can cause these mammals to become incidentally entangled, hooked, or otherwise captured. Additional risks to these species include the fisheries in Japan and the Lesser Antilles that specifically hunt pilot whales. These species are also exposed to injuries or death caused by inadvertent ship strikes (NOAA, 2017).

Some conservation efforts have been established to help mitigate the risk to pilot whales. NMFS created the Pacific Offshore Cetacean Take Reduction Plan, which was implemented in 1997. This plan established a California/ Oregon drift gillnet fishery requirement to use sound-generating pingers and six-fathom net extenders, which may reduce bycatch of cetaceans. NMFS also created the Pelagic Longline Take Reduction Plan, which became effective on June 18, 2009. This plan targets both regulatory and non-regulatory management measures. It also includes research recommendations to better understand marine mammal interactions incidental to the pelagic longline fishery (NOAA, 2017).

It can be argued that the current U.S. management of these pilot whales is as strong as possible. Currently, both species of pilot whales are listed as data deficient on the IUCN Red List (IUCN Red List, 2017). Conservation measures for these species are still needed and efforts are better focused in areas of fisheries interaction reduction.

Discussion

Objective One

The first objective of this capstone focused on providing a literature review with a marine-centric focus on aphanic species. This included a look at how species may be identified and an account of terms and descriptions used for aphanic species alongside a comparative look at published articles. We found that historically there have been many methods used to identify new species by way of morphological, DNA, and behavioral evaluations. Literature shows that each method can be controversial. When describing hidden species it has been revealed that there is not a standard term used to identify these species. Lastly, a Web of Science review of frequently used hidden species terms revealed that articles we expected to be included were missing. This was true for the foundational article describing aphanic species by Steyskal and the article by Shivji et al. on roundscale spearfish.

Objective Two

The second objective was to review current marine management agencies and legal framework and to provide a historical account of management examples through case studies. We found that management of ocean resources involves a wide range of U.S. and foreign agencies and stakeholders. Management is influenced by multiple factors including current and historical perspectives, conservation interests, and economics. Currently, management practices do not change when new aphanic species are identified. Management changes may be implemented when information is evaluated and stakeholders agree that there is enough justification for a change. The process to create change can be lengthy. Through the evaluation of case studies, we found that considerations to change the management of each aphanic species is situational. Management decisions for aphanic species may be based upon a number of factors such as the evaluation of current management practices, the trends and known pressures of each species, and the vulnerabilities of each species.

Objective Three

The third objective suggests best practices for using the information on aphanic species to provide effective management guidance. After evaluation of case studies, we found that immediate management changes are generally not made when there is a discovery of an aphanic species.

Conclusion

Objective One

In conclusion of objective one, multiple methods of species identification should be used when identifying a new species. A new species should not be classified as such by DNA or by morphology alone, particularly when considering aphanic species. When choosing a term to represent a newly-discovered hidden species, the use of the term aphanic can be used to describe all such species where additional biological information is unsubstantiated or not known. Inclusion of this term in the keywords of articles may aid in dissemination of information as it is being collected and identified. Other defining terms for hidden species should be included as key words when applicable. This information may help researchers to more readily identify existing articles involving any aphanic species. Additionally, researchers seeking historical information involving any topic should search for articles using a variety of key words to possibly reduce the chance of omitting relevant literature. This is emphasized for situations when researching aphanic species due to historical inconsistencies in terminology.

Objectives Two & Three

The second objective was to review current marine management agencies and legal framework and to provide a historical account of management examples through case studies. The third objective suggests best practices for using the information on aphanic species to provide effective management guidance. There are many agencies that work both together and independently to manage our ocean's resources. The ocean's resources may be better conserved by consistent policies and management practices across the globe, however this topic should be evaluated and addressed in a future study. Historical examples by way of case studies lead to the recommendation that management of newly-discovered aphanic species should remain unchanged until all information relating to species status has been collected. Information can then be reviewed by the appropriate stakeholders so management changes can be made as needed.

Future Recommendations

This review identified three additional items of focus. The first is that there should be an increase of research by scientists and naturalists to identify aphanic species and to subsequently gather as much information about them and their relationship to other species and then environment as possible. The second is to highlight the importance information transfer to governing bodies as necessary for evaluation and possible management changes. The third is to evaluate the historical relationships between all bodies that govern ocean resources and to suggest changes that could be implemented to improve consistency of conservation approaches across nations.

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