

Warming Temperatures have the Potential to Increase *Chondrilla nucula* Populations, Leading to an Increase in Hawksbill Sea Turtle (*Eretmochelys imbricata*) Abundance and Nesting Sites

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Introduction

Hawksbill sea turtles (*Eretmochelys imbricata*) are found in tropical and sub-tropical regions globally (**Fig. 1**) and are the largest spongivores on coral reefs, meaning they feed exclusively on sponges (Blumenthal et al., 2009; León and Bjorndal, 2002; National Oceanic and Atmospheric Administration, 2022). Sponges consist of either a calcareous or siliceous skeleton composed of structures called spicules. The morphology and arrangement of these spicules are unique to each sponge species (Łukowiak et al., 2013). Monaxons are a type of spicule that are needle-like in shape (**Fig. 2A**), whereas spherical spicules are sub-spherical in shape (**Fig. 2B**) (Andri et al., 2001; Cárdenas, 2020). Hawksbills feed substantially on sponges with spherical spicules (Łukowiak et al., 2013). As a result, these turtles affect the diversity and structure of coral reef ecosystems. Stony corals and sponges compete for space within the reef. 80% of the time, sponges outcompete these corals, and some sponge species are known to kill corals indirectly. *Chondrilla nucula* (Caribbean chicken-liver sponge) contains spherical spicules making it a significant part of the hawksbills' diet. (León and Bjorndal, 2002; Łukowiak et al., 2013) *C. nucula* and stony corals need open, rigid substrate to attach and grow, and shallow, well-lit areas; *C. nucula* is found at depths <30 m (Chelossi et al., 2007; León and Bjorndal, 2002). *C. nucula* outcompetes most coral species, making it the dominant competitor; it is fast-growing and can regenerate its tissues quickly after being wounded or consumed (Aronson et al., 2002; Rincon-Diaz et al., 2011; Swearingen III and Pawlick, 1998). It is considered a major threat to a reef in Belize and has resulted in >70% of all coral overgrowths in the Florida Keys, meaning the sponge has grown over living coral (Hill, 1998; León and Bjorndal, 2002). However, in the presence of hawksbills, a high coral abundance on Caribbean reefs is present. This is because *C. nucula* is the most common prey species found in hawksbills' diet and is the most nutritious out of all their prey species; predation from hawksbills prevents *C. nucula* from overgrowing (Rincon-Diaz et al., 2011).

Since 1600 AD, there has been a decline in sponges containing monaxial spicules and an increase in sponges containing spherical spicules. Since 1684, hawksbills have been decreasing and, as a result, were listed as critically endangered by the IUCN in 1996 (Beggs et al., 2007; Łukowiak et al., 2013). This began when the Europeans started hunting hawksbills for their meat in 1684, greatly reducing their population. During the next two centuries, however, the meat industry for turtles declined due to the toxic meat attributed to their diet. Some sponge species contain chemicals as a defense mechanism that hawksbills avoid if possible. Before hunting pressures, hawksbills were so abundant that they were forced to feed on both chemically defended and undefended sponges. The chemicals contained in the defended sponges resulted in their meat being inedible. Despite this, hawksbills were still being hunted for fat, and their eggs were harvested. In 1900, the meat of turtles was edible, suggesting that hawksbills were no longer feeding on chemically defended sponges due to a decrease in intraspecific competition. Therefore, the low population of hawksbills has allowed sponges with spherical spicules to increase (Łukowiak et al., 2013).

The increase in spherical sponges like *C. nucula* causes a decrease in coral species, but climate change also poses a threat to these corals. (Bell, 2018). As carbon dioxide (CO₂) levels increase in the atmosphere, the CO₂ taken up by the ocean also increases, resulting in a lower pH. An increase in CO₂ causes carbonic acid (H₂CO₃) to be formed, which reacts with carbonate

(CO_3^{2-}) to form bicarbonate (HCO_3^-) (**Fig. 3**). As a result, organisms such as coral that rely on calcium carbonate (CaCO_3) to make their shells can no longer do so since CO_3^{2-} is limited (Figuerola et al., 2021). In addition, warming temperatures are another threat to corals because they result in coral bleaching. When temperatures get too high, zooxanthellae are eliminated from corals, turning the corals white and killing them if conditions do not improve (Douglas, 2003). *C. nucula*, on the other hand, can tolerate warming temperatures and has survived an unusually high-temperature event resulting in coral bleaching in the Caribbean (Carballo and Bell, 2017). This is because *C. nucula* has a symbiotic relationship with *Synechococcus spongiarum*, a cyanobacteria (Usher et al., 2004). Instead of expelling this species like coral does zooxanthellae, *S. spongiarum* is more tolerant of increased temperatures and ocean acidification (Lee, 2012). Elevated sea temperatures have also caused an increase in diseases found in marine organisms in recent decades. In 1938, 70-95% of sponge species in the Caribbean and 60% of sponge species in the Ligurian Sea in 1987 died due to disease. That being said, *C. nucula* can defend against many disease-causing bacteria by damaging their cell wall (Webster, 2007). The ability of *C. nucula* to withstand warming temperatures can be advantageous to hawksbills (Hawkes et al., 2009).

Hawksbill populations have declined by 80% in the past three generations (Gane et al., 2020). In addition to hunting, hawksbills were also harvested for their shells to make jewelry. As a result, the number of nesting females decreased, leading to conservation efforts; in the mid-1970s, these turtles were protected under the U.S. Endangered Species Act. Many monitoring programs were put in place by the early 1980s focusing on nesting sites; researchers monitored nesting females, egg numbers, and hatchling success. Today, common threats include direct take, fisheries bycatch, global warming, pathogens, and pollution, with coastal development and nest predation having the most impact (**Fig. 4**) (Donlan et al., 2010). Developed beaches, such as those for tourism, decrease the preferred vegetation that hawksbills use to nest. Other hazards like sea walls, pavements, and other anthropogenic structures prevent them from reaching the higher levels of the beach. This causes hawksbills to build their nests in the reef/rubble substrate instead of the preferred sandy substrate. The reef/rubble substrate supports a higher species diversity than the sandy substrate, making their nests more vulnerable to predators. Artificial lighting also deters them from nesting and causes the hatchlings to be misoriented when entering the water. In addition, they become exhausted and dehydrated, are more exposed to predation, and use a less efficient swimming style (Harewood and Horrocks, 2008).

Protecting these nesting sites is imperative, especially in the face of climate change. Conservation techniques have been done to help retain sea turtle nesting sites. Predation by ghost crabs, genus *Ocypode*, consume 90-100% of hatchlings in a nesting site on Cousine Island, making this a considerable threat to their populations. In addition, the sex of sea turtles is determined by temperature: females develop at higher temperatures, and males develop at lower temperatures. Even a 1-2 °C change in temperature can cause a difference in the sex ratio. 29.2 °C is the pivotal temperature that determines males versus females. Global warming has resulted in more females being hatched (Gane et al., 2020); the standard ratio of female to male is 1:1, but warming temperatures have caused 2.4-4 females to be hatched for every male (Hawkes et al., 2013). Gane et al., 2020 used fences (**Fig. 5A**) and nettings (**Fig. 5B-C**) to help protect hawksbill eggs from crab predation and found that fences provide better protection from predators, while nettings had a higher hatch success and were skewed towards male development. The use of netting could be used to not only protect hawksbill hatchlings from predation, but to alter the

temperature to favor more male hatchlings, which is imperative as global warming continues to increase.

Since hawksbills are critically endangered, it is vital to understand how warming temperatures will affect this species. Coral bleaching and the inability of corals to calcify have already occurred due to global warming, potentially causing *C. nucula* to increase in abundance in these reefs. This could be beneficial to hawksbills, as it creates a potential food source for them which, in turn, could increase nesting sites. It has been shown that some areas with poor reef health have increased in hawksbill nesting sites (Hawkes et al., 2009). It is also possible that the diversity within coral reef ecosystems will increase and be skewed toward organisms that can survive with warmer temperatures and ocean acidification. Bell et al., 2018 showed that some sponge species show positive effects with increased ocean acidification (**Fig. 6A**) and ocean warming (**Fig. 6B**). The grazing on *C. nucula* by hawksbills could allow heat-tolerant species to grow and expand, possibly increasing biodiversity despite losing key coral species.

This study will focus on the Cayman Islands, specifically Grand Cayman and Little Cayman (**Fig. 7**). The Cayman Islands are well-enforced marine protected areas with little to no fishing allowed (Loh and Pawlik, 2014). In 2009, there was a massive coral bleaching event where 54 out of 69 coral species were affected, with 25 becoming bleached due to extreme temperatures (van Hooidonk et al., 2012). The range of temperatures most tropical corals can withstand is 18-30 °C (van Hooidonk et al., 2012). When the temperature is greater than 30 °C, coral bleaching can occur; *Siderastrea sidereal* (round starlet coral) was one the first coral species to bleach in Little Cayman when the temperature was 30.5 °C and was an early indicator of reef stress (Banks and Foster, 2016). Although there has been some recovery since then, it takes coral communities 10-30 years to recover, and bleaching events in Little Cayman occur every five years and are expected to occur annually by 2040, not allowing coral species to recover; the frequency and intensity of these disturbances are expected to increase with warming temperatures (Foster and Foster, 2018). This suggests that the abundance of *C. nucula* will increase due to more space availability and their ability to survive in these harsh conditions, which, in turn, will increase hawksbill abundance and nesting sites. In addition, other sponge species that are tolerant of warming temperatures may also increase, creating more food sources for hawksbills (**Fig. 6**) (Bell et al., 2018).

Due to low population numbers, hawksbill nests in the Cayman Islands were very low in the late 1900s. Blumenthal et al., 2021 performed a study from 1998-2019 looking at sea turtle nests in the Cayman Islands and found that Little Cayman had more nesting sites than Grand Cayman and had increased over two five-year periods. Nests in Grand Cayman, on the other hand, remained low in the last five years of the study. These data are consistent with a higher abundance of nesting sites in areas with poor reef health. This study aims to continue this research to see if the nesting abundance continues to increase at Little Cayman and remain the same at Grand Cayman to determine if there is a correlation between warming temperatures and increased food availability.

Objectives

The objectives of this study are to look at whether water temperature affects hawksbill sea turtle prey, thus affecting hawksbill and nesting abundance using four unhealthy reef ecosystems at Little Cayman, two of which are no-take marine reserves (**Fig. 8A**), and four healthy reef ecosystems at Grand Cayman (**Fig. 8B**) as study sites. Unhealthy reefs for this study are reefs

that show signs of coral bleaching, and the water temperature is above 30 °C. Healthy reefs are reefs with no signs of coral bleaching and water temperatures between 18-30 °C.

This will be done by

- Finding and counting the number of nests at each site to determine hawksbill nest abundance
- Performing aerial surveys to count the number of hawksbills at each site to determine hawksbill abundance
- Engaging in line transect surveys to
 - Identify and count sponge and coral species at each site to determine the sponge and coral diversity
 - Take water temperature measurements at each site to determine if temperatures exceed 30 °C

Methods

This study will take place over a five-year period to see if temperatures continuously increase and, if so, how that affects the data.

Nesting abundance- Beach walks will be performed twice weekly during the nesting season from May-September at both islands (Bell et al., 2007). Researchers will walk along beaches where known nesting sites are (**Fig. 9**). Hawksbill tracks will be used to help identify nests (**Fig. 10**). If tracks are found but no nest, it will not be counted. After finding a nest, tracks will be erased to avoid recounting of nests (Marco et al., 2012). The number of nests will be compared between islands.

Hawksbill abundance- Aerial transect surveys will occur twice a month every month. The plane will fly around each island and count the number of hawksbill sea turtles seen. The transects will extend from the shoreline to 140 km. Transect data will be entered into the program Distance 6.0 to estimate turtle abundance for each site (Seminoff et al., 2014). This data will then be compared between islands.

Coral and sponge abundance and species diversity- Four sample sites for each island will be sampled each month (**Fig. 8**). The abundance of corals and sponges will be determined at three depth ranges: 3-6 m, 7-12 m, and 13-18 m. At each depth, sponge and coral species will be counted and identified at each site up to the species level, if possible, by using a 1 m x 1 m quadrant. This will be repeated until 10 m is reached, alternating between the right and left sides of the measuring line (Bruckner, 2010). Species will then be compared between islands to see which island has a higher abundance of sponges, specifically *C. nucula*.

Water temperature- Water temperature will be tested when performing line-transect surveys at each site. The water temperature will be taken using temperature probes, and two readings will be sampled before, during, and after line-transect surveys for a total of six readings. Temperatures will be compared between each site.

Comparison between sites- Each set of data collected will be compared between sites at each island, between sites with and without no-take marine reserves at Little Cayman, and between years. To do this, the data will be analyzed using an ANOVA test. A Kruskal-Wallis test will be

used if ANOVA assumptions (normality or homogeneity of variance) are not met. A Tukey's honestly significant difference test will be used if the results are significantly different (Bézy et al., 2015; López-Castro et al., 2010).

Significance

Hawkes et al., 2009 mentioned how increases in hawksbill nesting sites, despite poor reef health, could reflect an increase in food availability and should be further studied. This study aims to further their research on how climate change affects marine turtles. This research is significant because global warming not only affects sea turtles and marine ecosystems, but it is intensifying at an alarming rate. Water temperature data will determine if water temperatures are continuing to increase, causing more frequent and intense coral bleaching events, and allowing *C. nucula* to expand. By looking at reef diversity, this survey will show if there is a greater abundance of *C. nucula* in coral bleached areas and, in turn, can show if there are more hawksbills present. This data will also see if the no-take marine reserves show a higher abundance of hawksbills than the sites outside the reserves. This can allow conservationists to create more marine reserves if they prove to be effective in protecting hawksbill populations. This could also generate discussion as to whether conservationists want to try and restore the natural coral reef ecosystem or allow for *C. nucula* to grow if hawksbill populations are increasing. The results can also bring about further research like monitoring water temperatures at Grand Cayman to see if coral reefs start to succumb to bleaching events as global warming continues.

In addition, this work will help to determine if nesting sites are increasing in areas of high *C. nucula* abundance, which is imperative to hawksbill populations. This can allow conservationists to eliminate or greatly reduce threats to nesting sites such as coastal development and artificial lighting. Coastal development should not occur in areas of high nest abundance, and artificial lighting should not be used during the nesting season. As an alternative, lights that are safe for turtles should be utilized to reduce misorientation in sea turtle hatchlings. Other conservation techniques such as using nets to help balance out the sex ratio of hawksbills could also be applied to these high nesting areas because as temperatures continue to increase, less and less males will be hatched, which will lower reproductive success in the future.

Since hawksbills are found in all the world's major oceans and coral bleaching is occurring globally, the results of this study can be applied to other areas where warmer temperatures are occurring (National Oceanic and Atmospheric Administration, 2022). This can allow management practices in areas with a high abundance of hawksbills as they are highly migratory species (Blumenthal et al., 2009). Knowing where there are high abundances of *C. nucula* in other areas can allow conservationists to protect those areas, as well as nearby nesting sites, if this study finds high abundances of hawksbills in these areas. This can enable management actions such as modifications to fishing gear to reduce entanglement; turtle excluder devices (TEDs) (**Fig. 11A**) and deterrents like lights or chemical repellents (**Fig. 11B**) have been shown to reduce sea turtle bycatch and have no impact on target catch (Vasapollo et al., 2019; Wang et al., 2010).

Overall, this study will help analyze critical habitats for hawksbill sea turtles, both nesting and foraging sites, which can determine where conservation efforts need to be focused to save these critically endangered sea turtles, and implicates that global warming may be beneficial to these species if other threats are reduced.

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Figures

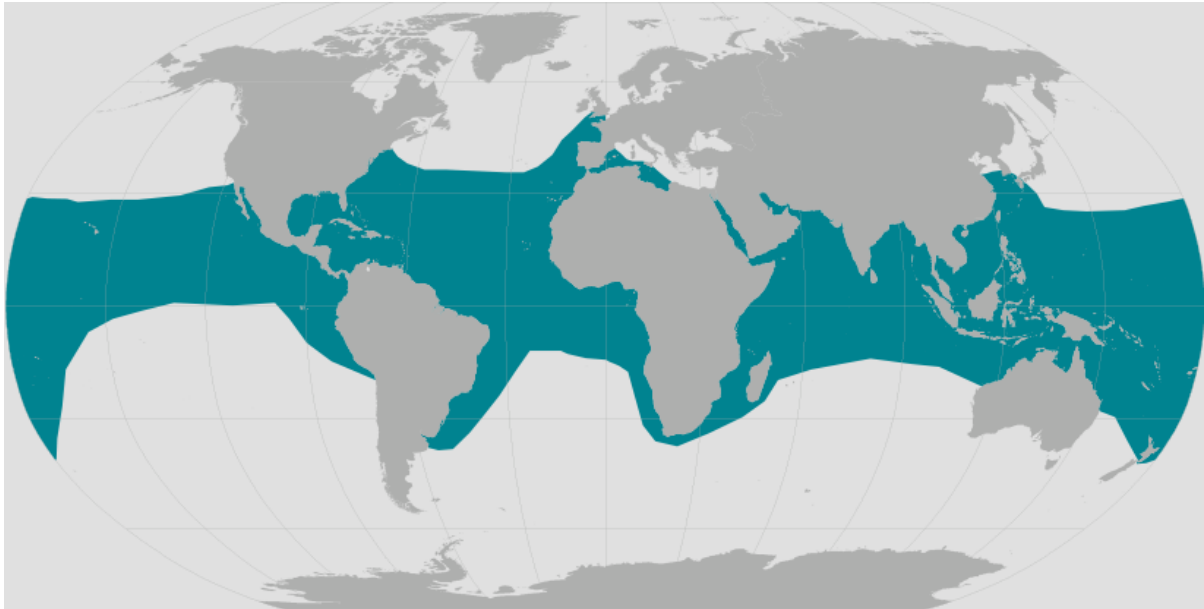


Figure 1. Hawksbill sea turtle distribution (National Oceanic and Atmospheric Administration, 2022).

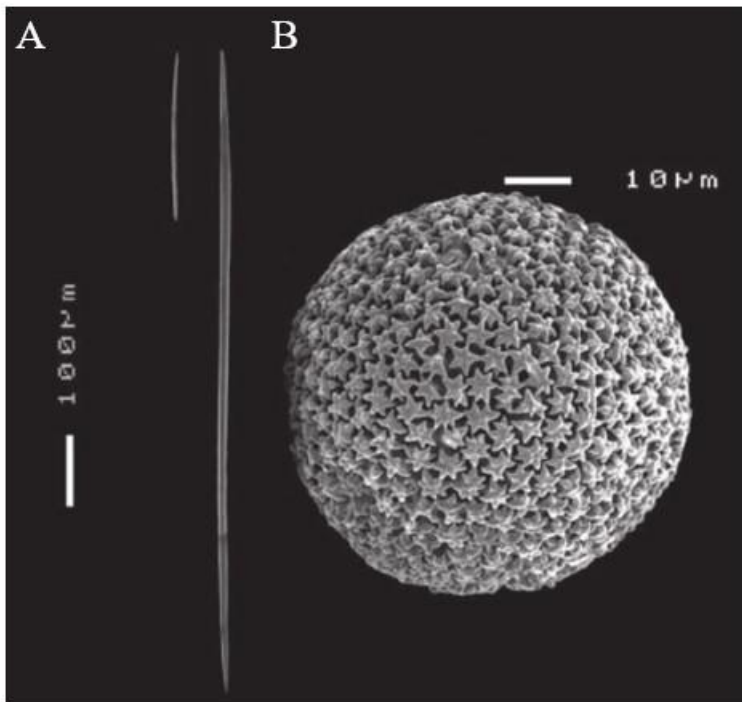


Figure 2. Sponge spicules. (A) Monaxon spicule. (B) Spherical spicules (Van Soest and Beglinger, 2008).

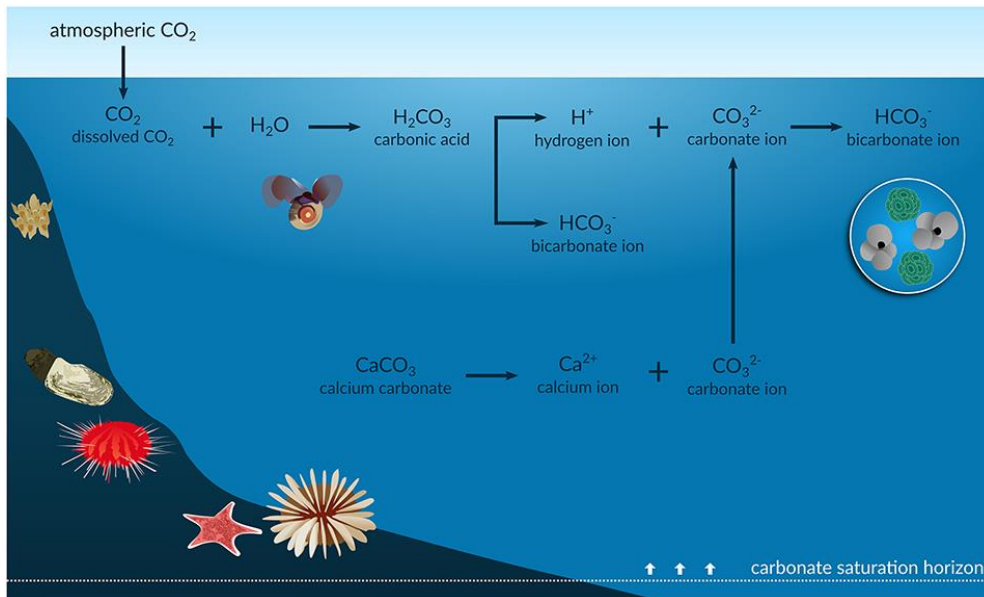


Figure 3. Infographic of the ocean acidification process. The anthropogenic CO_2 absorbed by the oceans increases the concentration of hydrogen ions (H^+) and in bicarbonate ions (HCO_3^-) and a decrease in carbonate ions (CO_3^{2-}). The reduction in CO_3^{2-} is shallowing the carbonate saturation horizons, with potential impacts on shells and skeletons of marine calcifiers such as foraminifera, corals, echinoderms, mollusks, and bryozoans (Figuerola et al., 2021).

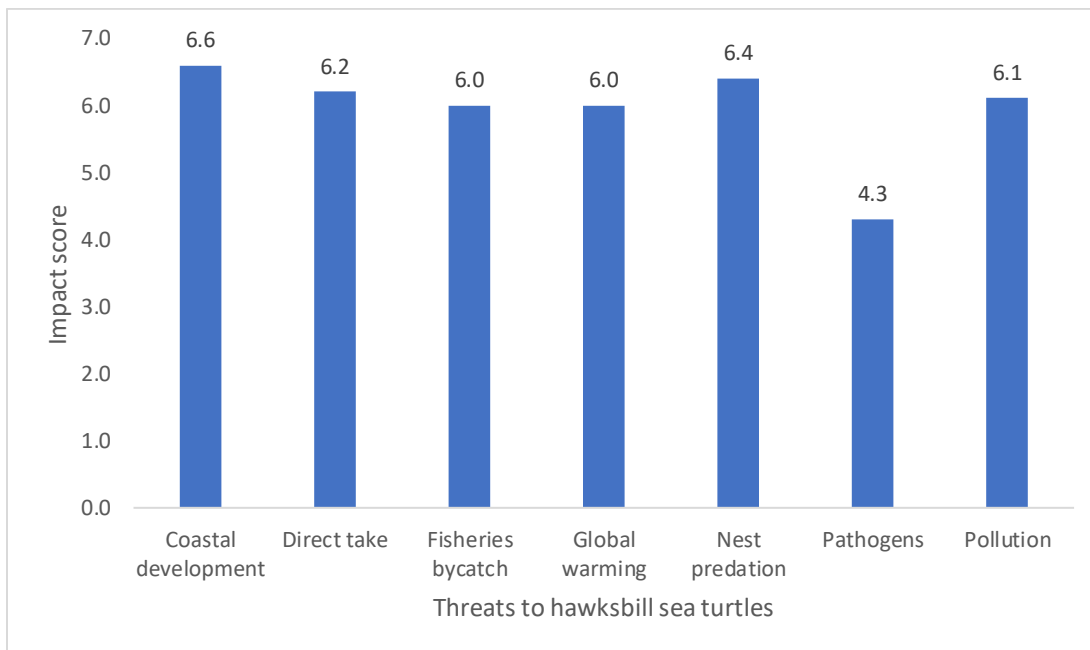


Figure 4. Impact scores for each threat for hawksbill sea turtles. Impact scores follow International Union for Conservation of Nature/Birdlife International scoring scheme: 0–2, no or negligible impact of hazard; 3–5, low impact; 6–7, medium impact; 8–9, high impact (Donlan et al., 2010).



Figure 5. Management methods used to protect hawksbill sea turtle nests from predation at Cousine Island, Seychelles: (A) Fencing- plastic fencing was placed inside the hole around the nest. (B) Netting- the eggs were carefully placed within the netting and then (C) covered in sand (Gane et al., 2020).

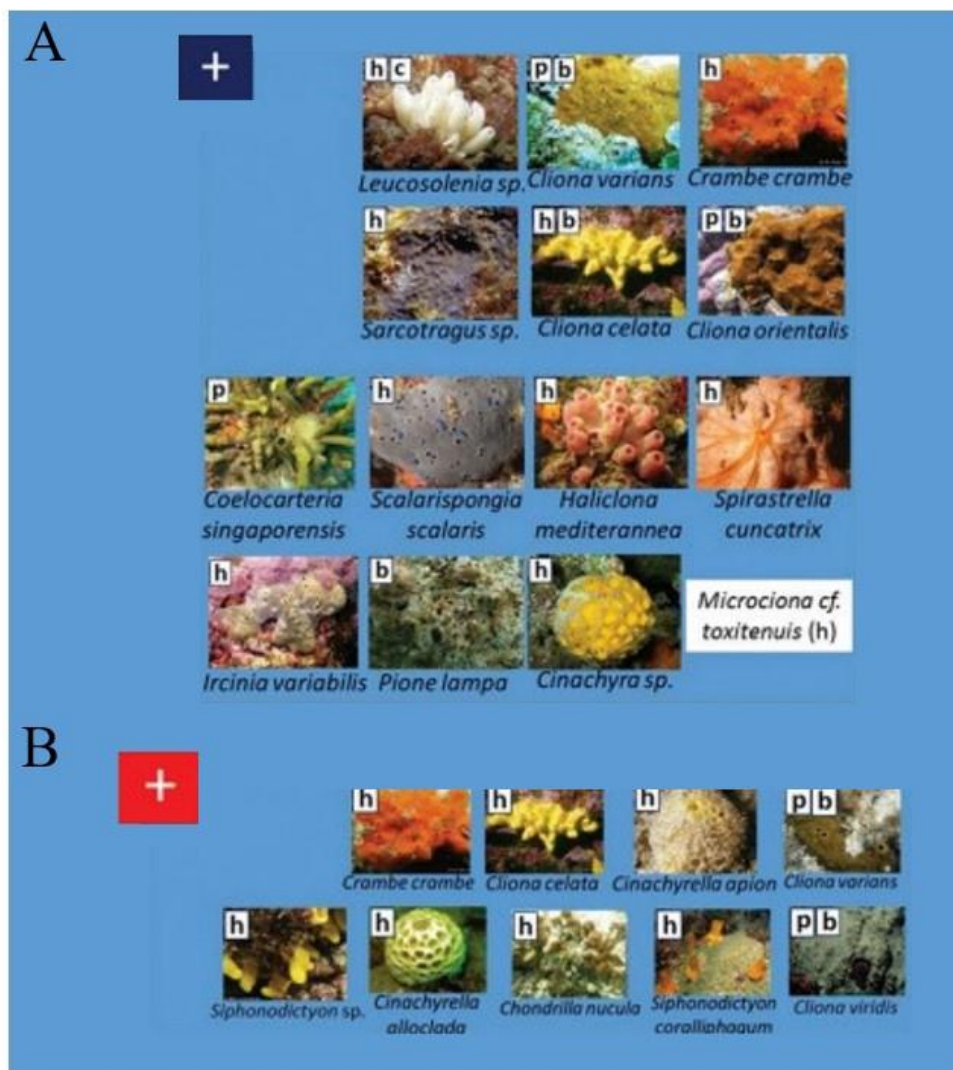


Figure 6. Summary of the number of sponges showing positive (+) effects in response to (A) ocean acidification, (B) ocean warming (Bell et al., 2018).

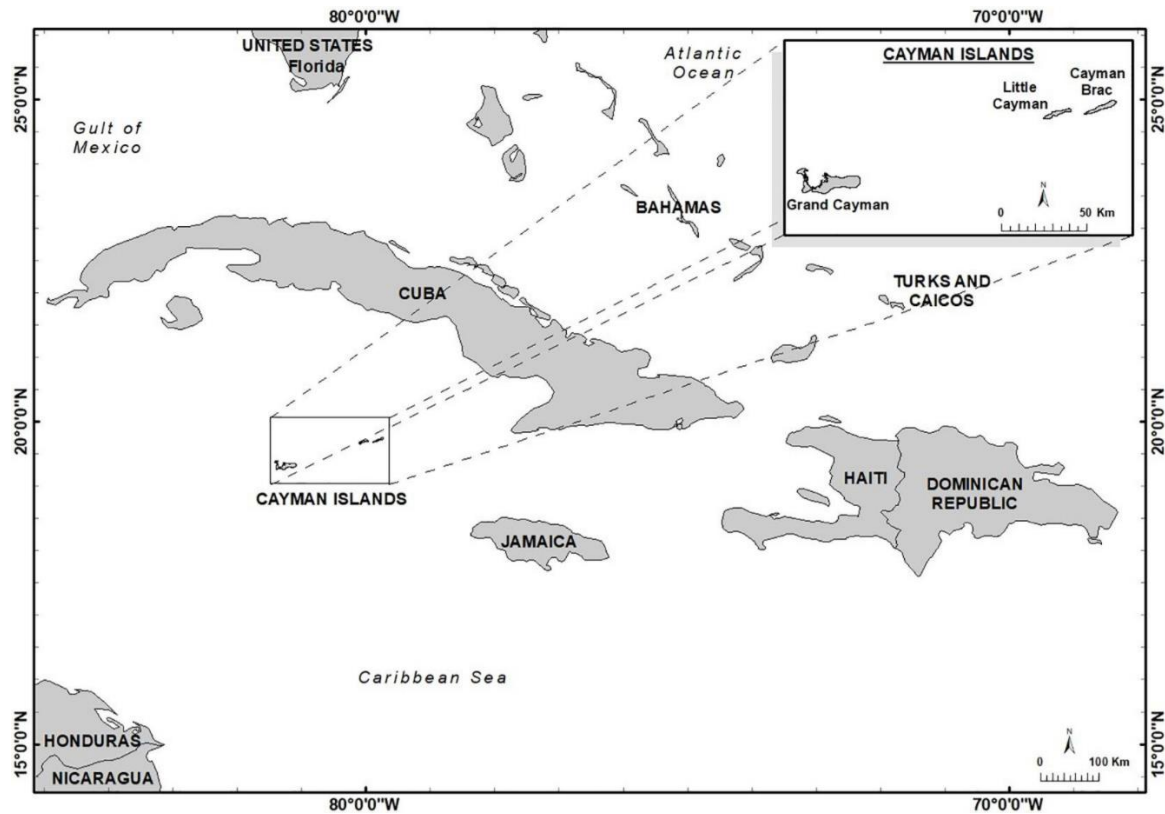


Figure 7. Location of the Cayman Islands in the Caribbean Sea showing study sites: Grand Cayman and Little Cayman (Blumenthal et al., 2021).

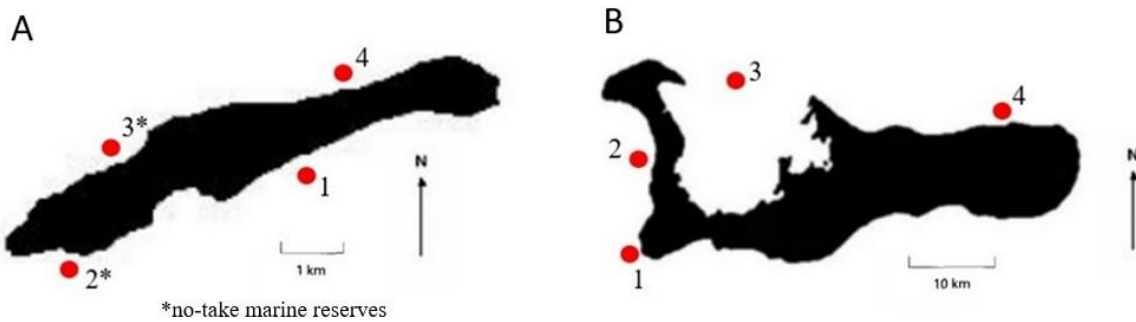


Figure 8. Map of (A) Little Cayman and (B) Grand Canyon showing sampling sites (Bruckner, 2010; Coelho and Manfrino, 2007).

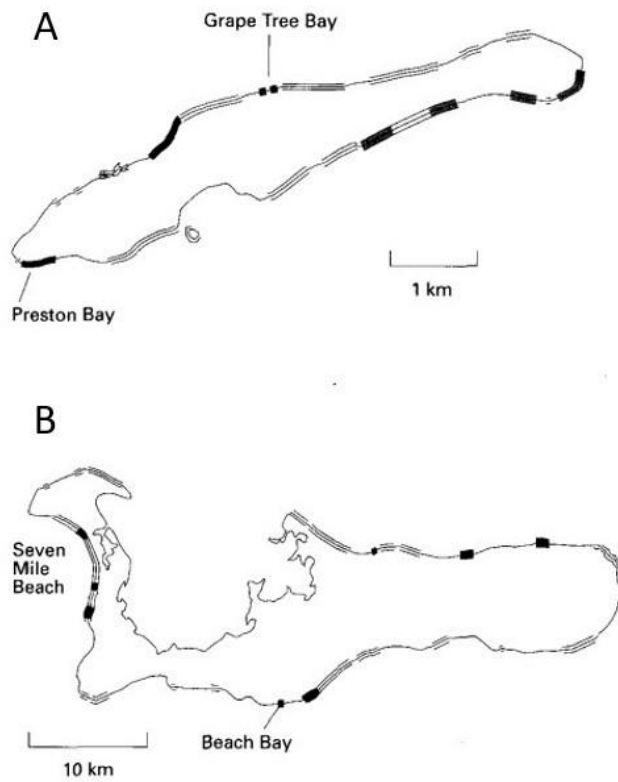


Figure 9. (A) Little Cayman (B) Grand Cayman, showing habitat suitable for hawksbill turtle nesting (Aiken et al., 2001).

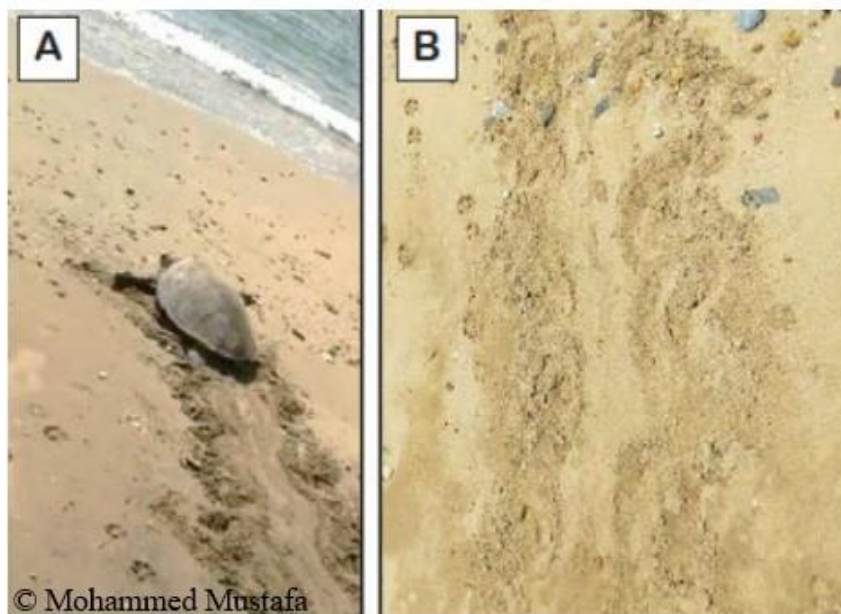
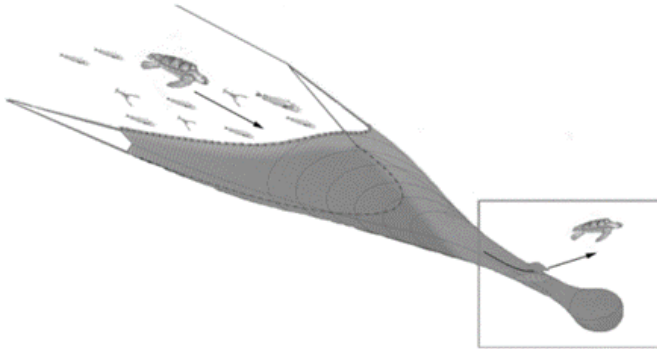


Figure 10. (A) Hawksbill sea turtle crawling to the sea after its nesting attempt. (B) Hawksbill sea turtle tracks (Yaghmour and Jarwan, 2020).

A



B

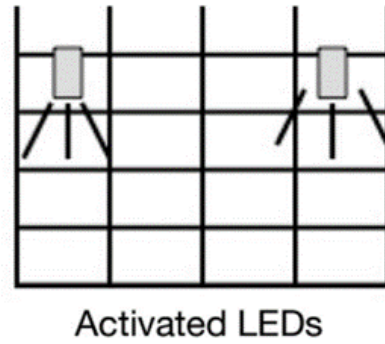


Figure 11. Gear modifications to reduce turtle bycatch. (A) Turtle excluder device (TED). (B) Activated LED lights or chemical lightsticks placed on gill nets used to deter sea turtles (Vasapollo et al., 2019; Wang et al., 2010).