

**Biology, monitoring, and management of a
tropical marine gastropod:
the Queen conch (*Lobatus gigas*) in the Caribbean**



Erik Maitz Boman

Propositions

1. The patchy distribution pattern of adult conch (*Lobatus gigas*) is caused by gregarious behavior and not related to environmental variables. (this thesis).
2. The main threat to the long-term survival of the queen conch (*Lobatus gigas*) is the rapid spread of an invasive seagrass species in the Caribbean region. (this thesis)
3. Global warming will increase the urgency for the continuous re-evaluation of life-history traits of exploited ectotherm species.
4. Vaccines do not cause autism.
5. The main threat to marine life and the sustainable use of marine resources on St Eustatius is the inevitable closing-down of the islands oil storage facility.
6. Expecting and accepting the unexpected is a must to survive on St Eustatius.

Propositions belonging to the thesis entitled:

Biology, monitoring, and management of tropical marine gastropod:
the Queen conch (*Lobatus gigas*) in the Caribbean

Erik Maitz Boman

Wageningen, 4 September 2019

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Biology, monitoring, and management of a tropical
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För Anna, Mio och Aston, jag älskar er mer än mest.

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Chapter 1

Biology, monitoring, and management of a tropical marine gastropod: the Queen conch (*Lobatus gigas*) in the Caribbean:

General Introduction

Erik Maitz Boman

Introduction 1.1

Marine gastropod fisheries represent a relatively small amount (<1 %) of global marine fisheries production (FAO 2011-2019). However, these fisheries often have high economic and cultural value locally, such as in the Caribbean, due to their importance for local consumption and their high value in the international market (Theile 2001, Levia & Castilla 2002). The large marine gastropod queen conch (*Lobatus gigas*), found throughout the Wider Caribbean Region (Figure 1.1), supports one of the most important fisheries in the region (Appeldoorn 1994, Theile 2001).



Figure 1.1. The approximate distribution range of queen conch (*Lobatus gigas*) (blue area) in the Wider Caribbean Region.

Queen conch are particularly vulnerable to over-fishing due to their unique life history (planktonic phase, slow-moving, density-dependent survival, and reproduction) (Appeldoorn et al. 2011). The exploitation of queen conch throughout large parts of its natural range (Stoner 1997) has led to population collapses (e.g. Florida) and decrease in population densities to levels where mating success is affected (Delgado & Glazer 2007, Stoner et al.

2012a, Stoner et al. 2018). Due to concerns for the exploitation of the species, as well as the degradation of conch habitats, certain measures have been taken. These include the listing of queen conch to Appendix II of CITES in 1992 and the establishment of the International Queen Conch Initiative, which aims at improving regional management through harmonized regulations (Appeldoorn et al. 2011). However, current regulation such as minimum size and a closed season is often based on outdated biological information and inappropriate biological metrics to measure maturity (Stoner et al. 2012a). Despite the implementation of management actions combined with a more stabilized conch production over the last decade, the recovery of already overfished conch populations has been slow (Theile 2001). Furthermore, degradation of nearshore conch habitats due to land-based human activities has in some cases led to breeding failure (Ducrot et al. 2007, Glazer et al. 2008) and decreased survival and growth of conch juveniles and larvae (Sanders 1984, Orth et al. 2006). A more recent threat to conch with the potential of altering and possibly degrading already afflicted conch habitats, in particular native seagrass beds, is the introduction of the invasive seagrass *Halophila stipulacea* to the Caribbean. This invasive seagrass has been demonstrated to have a negative effect on native seagrass beds (Willette & Ambrose 2012, Scheibling et al. 2018), which in many locations are essential for juvenile conch (Stoner 2003). Existing knowledge regarding the possible effects of *H. stipulacea* on conch is minimal. However, the consequences could be severe if conch habitats experience further degradation from this rapidly spreading invasive species (Vera et al. 2014, Willette et al. 2014), which would likely lead to reduced population growth and lower resistance to fishing mortality for already strained conch populations.

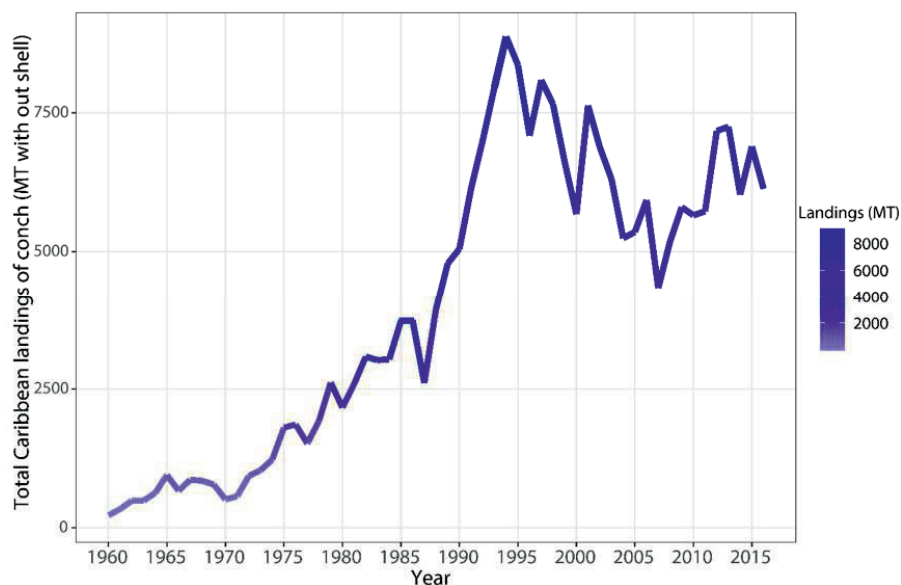


Figure 1.2. Total reported landings (metric tons = MT) of queen conch (*Lobatus gigas*) in the Caribbean. Landing values reported in meat weight (unclean/dirty meat) using 5.3 conversion factors from total weight with shell (FAO 2011-2019). Landings presented show a trend in conch production reported by nations to FAO and are not entirely comparable to total estimated landings (7800 MT) by Prada et al. (2017) which is based on more complete data set not available for the entire time series presented here.

For effective management of these fisheries, a good understanding of the population dynamics of the targeted species as well as effective monitoring is paramount. Although queen conch has been researched extensively, essential knowledge of life-history traits such as size-at-maturity, habitat preference, population connectivity, potential effects of invasive species, as well as practical tools to monitor the species throughout its entire depth range is lacking. The main objectives of this study were, therefore, to address knowledge gaps of the biology and ecology of the queen conch so that the distribution and dynamics of this species in relation to its environment are better understood. Such information will further improve our knowledge of marine gastropod biology in general, as well as our understanding of the effects of direct and indirect human-induced pressures on queen conch in the Caribbean. In addition, this study aimed to tackle some methodological shortcomings in the surveying and assessment of queen conch. Ultimately, these findings could be instrumental in the management and conservation of the species.

In the following sections, the main subjects addressed in this dissertation are described, and crucial knowledge gaps related to each subject are delineated.

1.2 Queen conch fishery and management in the Wider Caribbean Region

In the Caribbean, queen conch is commercially exploited in at least 22 countries, with annual landings of approximately 7800 MT and an estimated value of about USD 74 million (Chakalla & Cochrane 1996, FAO 2015, Prada et al. 2017). Although the fishery contributes to a relatively small part of the economy in the region overall, it is still the second most important benthic fishery and is only surpassed by the spiny lobster (*Panulirus argus*) fishery (Appeldoorn 1994, Theile 2001). In the 1970s the commercial harvest of queen conch began to increase dramatically due to a demand from the international market (Theile 2005). The harvest of conch peaked in the 1990s at a level of almost ten times that of two decades earlier (FAO 2011-2019) (Figure 1.2, 1.3, 1.4). The increased exploitation of queen conch throughout large parts of its natural range (Stoner 1997) has led to population collapses (e.g. Bermuda, Florida) (Berg et al. 1992a, Glazer & Berg 1994, Berg & Glazer 1995), and reduction of population densities to a point (< 100 adult conch/ha) where mating success can be negatively affected (e.g. Bermuda [Berg et al. 1992a], the Bahamas [Stoner et al. 2018], Dominican Republic [Delgado 1999], Florida [Berg et al. 1992b, Berg & Glazer 1995] Guadalupe [Chalifour 2010], Puerto Rico [Mateo et al. 1998]). The decrease in landings of queen conch since its peak in the 1990s has been attributed to overfishing (e.g. Florida [Berg & Glazer 1995], Bermuda [Berg et al. 1992a]). However, temporary closure of some fisheries (e.g. Dominican Republic, Haiti, Honduras) (CITES 2003) as well as stronger management and conservation efforts (e.g. Pedro Bank, Jamaica), which aimed to promote a more sustainable and productive fishery, also contributed to the decrease in landings (Prada et al. 2017). After a sharp decline in conch catches in the late 1990s and early 2000s, the harvest of conch has stabilized in the last decade, and some nations currently operate under sustainable harvest quotas, which maintain high densities that ensure mating success (e.g. Belize [Azueta 2012], Jamaica [Morris 2015], Nicaragua [Navarro 2012]). Although improvements have been documented in some conch stocks in the Caribbean in terms of increased densities and more stable catches, many conch populations in heavily fished areas still have densities well below sustainable levels (< 100 adult conch/ha) and in

some cases are experiencing further declines due to fishing (e.g. the Bahamas [Stoner et al. 2018], Barbados [Vallès & Oxenford 2012], Cuba [Cala et al. 2012], Guadeloupe [Patin et al. 2013], Haiti [Wood 2010], Mexico [Cala et al. 2013]). Furthermore, the recovery of already overfished conch populations such as Bermuda (Berg et al. 1992a) and Florida (Berg & Glazer 1995) has been slow and despite a complete closure of the conch fishery in 1978 and 1985 respectively, stocks have yet to fully recover (Barrett 2000, Glazer & Delgado, 2003).

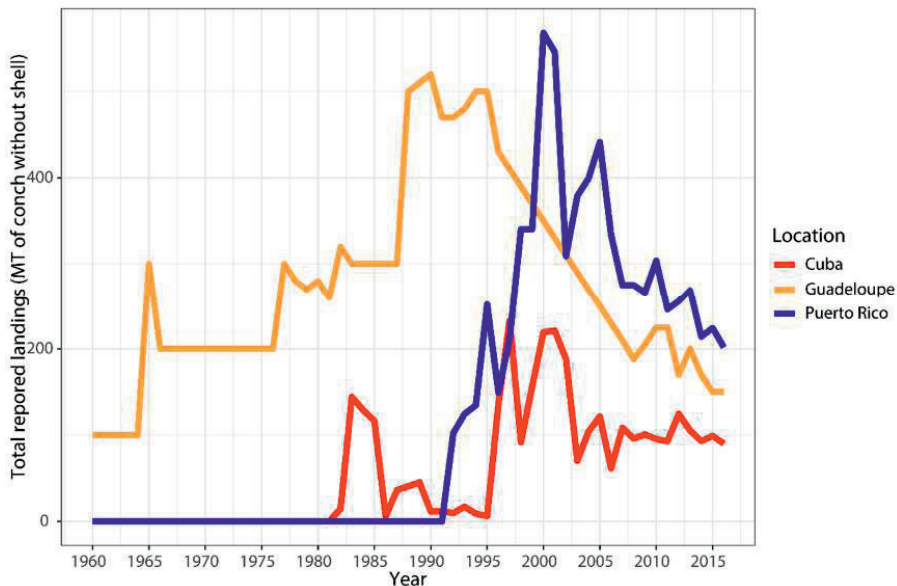


Figure 1.3. Total reported landings (metric tons = MT) of queen conch (*Lobatus gigas*) for three nations (Cuba [red], Guadeloupe [orange], Puerto Rico [blue]), in the Caribbean with a current negative trend in landings. Landing values reported in meat weight (unclean/dirty meat) using 5.3 conversion factors from total weight with shell (FAO 2011-2019).

The main product of the queen conch trade is the white meat, which was once a locally important source of protein in many parts of the Caribbean, but is now mainly exported to foreign markets (Theile 2005). Nevertheless, conch pearls, shells and other parts of the conch are also traded but to a lesser degree than the meat, although the total extent of their trade is mostly unknown (FAO 2015, Prada et al. 2017). The queen conch fishery is largely artisanal although an industrial fishery is also present. It supports full or partial incomes for approximately 20,000 fishermen (FAO 2015, Prada et al. 2017) and makes up about 10 % of the marine production in the Caribbean (FAO 2014).

Traditional techniques such as free-diving and bottom gill-nets are used in many artisanal queen conch fisheries, while more modern methods such as scuba-diving and hookah involving compressed air are most common in the industrial conch fishery. Fishing techniques involving compressed air have also allowed fishermen to target conch populations at greater depths (Theile 2001).

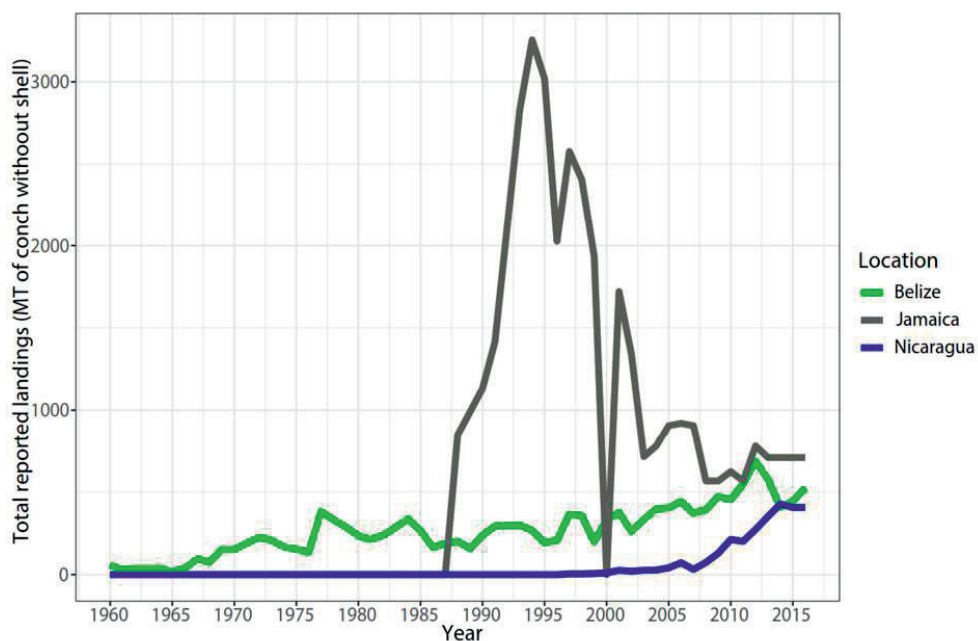


Figure 1.4. Total reported landings (metric tons = MT) of queen conch (*Lobatus gigas*) for three nations (Belize [green], Jamaica [yellow], Nicaragua [blue]), in the Caribbean with a current stable trend in landings. Landing values reported in meat weight (unclean/dirty meat) using 5.3 conversion factors from total weight with shell (FAO 2011-2019).

The artisanal part of the fishery uses small vessels (ca. 7-10 m) with a limited crew of divers (1 - 5 people) powered by outboard engines or sail and oars, limiting the fishing effort to day trips (Theile 2001). The more industrial fishery, on the other hand, uses larger vessels (up to 40 m), which can carry more than 40 divers and make week-long fishing trips (Theile 2001). Most artisanal fishery occurs on nearshore shallow banks or the narrow shelf which surrounds many islands in the Caribbean (e.g. Antigua and Barbuda, Saint Eustatius, Saint Lucia, Saint Vincent and the Grenadines), while the industrial fishery is concentrated on offshore banks (e.g. Jamaica, Belize, Colombia, Honduras, Turks and Caicos) (Theile 2001). To increase the

protection of queen conch stocks, most nations in the Caribbean have implemented minimum harvest size regulations for queen conch (Theile 2005), although these are often based on outdated and limited biological information as well as an inappropriate biometric indicator for maturity (i.e. shell length) (Stoner et al. 2012a). To set sustainable harvest quotas and regulate the effective amount of harvested conch, accurate estimations of adult conch densities, in shallow and deep areas alike, are essential. Moreover, accurate estimates are essential for maintaining densities at a level where successful reproduction can be maintained (Stoner & Ray-Culp 2000). Surveying of queen conch can, however, be logistically demanding and relatively expensive, particularly when deep (> 25 m) and remote offshore areas are surveyed (Queen Conch Expert Workshop Group Report 2012). This is due to the safety limitations of conventional survey methods using scuba. Areas below 20 m are thus rarely surveyed, and areas below 30 m are generally excluded from biomass estimates (Queen Conch Expert Workshop Group Report 2012, MRAG 2013). The development of new survey tools that can survey queen conch throughout the species' entire depth range (0 – 60 m) and accurately estimate live adult conch densities, without an increase in costs or risks to field staff, are thus needed.

1.3 Reproductive biology

Reproduction rate and success are important factors governing population dynamics, which is expressed via the classic equation, population size (N) = births (B) + immigration (I) – deaths (D) – Emigration (E). For queen conch, reproductive rate and success (B) are largely density-dependent, and an Allee effect in queen conch has been demonstrated, where reproductive activities begin to decrease below levels of 100 adult conch/ha and stop completely when densities drop to 50 adult conch/ha (Stoner & Ray-Culp 2000). Thus, successful reproduction can only occur at sufficient densities of sexually mature individuals. The ability to distinguish adults from sexually immature individuals, as well as knowledge regarding when individuals and approximately 50% of the population, become sexually mature (i.e. age or size at maturity), is essential. This is to ensure that mature conch densities remain above the critical density threshold (> 100 conch/ha) so that successful reproduction can occur.

In the Wider Caribbean Region, the queen conch reaches sexual maturity at ca. 3.5–4 years old (Appeldoorn 1988, Stoner et al. 2012a). After reaching its maximum length, the edge of the shell flares outward and develops a lip (Figure 1.5) which increases in thickness prior to, and also after the onset of maturity (Appeldoorn 1988, Stoner et al., 2012a). The shell stops growing in length before the conch reaches maturity, therefore shell length is not a reliable indicator of maturity. Despite this, much of the minimum size regulation among Caribbean nations is based on shell length. Lip thickness is, however, a more appropriate index of maturity than shell length (Stoner et al. 2012a, Foley & Takahashi 2017). Growth in lip thickness varies between different locations and has been found to be dependent on sex, depth, latitude, temperature, food availability, age class, and shelter (Appeldoorn 1988, Stoner & Sandt 1992). Recent studies have assessed the relationship between sexual maturity and lip thickness for both sexes using microscopic gonadal characteristics (Avila-Poveda & Cárdenas 2006, Bissada 2011, Stoner et al. 2012b). However, the research group involved in this project has found strong indications that the current information on size-at-maturity in relation to lip thickness for the Caribbean is based on inappropriate statistical methods and in urgent need of correction. As size-at-maturity is an important factor influencing reproductive success and population dynamics of a species, accurate knowledge regarding such life history traits is vital for the management of the fishery.

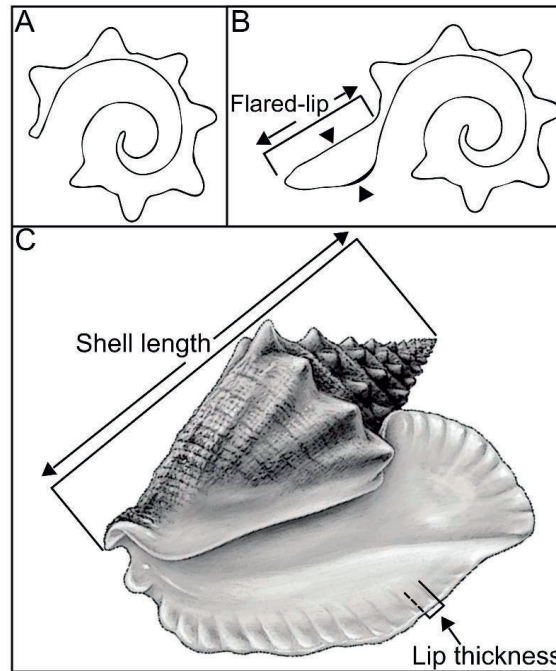


Fig 1.5. Cross-section of the shell of an immature (subadult) queen conch (*Lobatus gigas*) without a developed lip (A). Cross-section of a mature (adult) queen conch (*Lobatus gigas*) with a developed flared-lip. Opposing arrows represent the position where lip thickness is measured. (B). The shell of a mature queen conch (*Lobatus gigas*), with measured dimensions of shell length (SL) and lip thickness (LT) (C).

Another critical aspect influencing reproduction is the reproductive season, which in queen conch differs among locations in duration, although it generally occurs from March to October across the Caribbean. It also varies in intensity (i.e. the relative frequency of mature individuals engaged in reproductive behavior), which generally increases with a shorter duration of the reproductive season (Avila-Poveda & Baqueiro-Cardenas 2009, Aldana-Aranda et al. 2014). The duration and intensity of the reproductive season can be influenced by factors such as photoperiod, seawater temperature, and fluctuations in seawater temperature (Brownell 1977, Stoner et al. 1992, Aldana-Aranda et al. 2014). The duration of the reproductive season has been observed to fluctuate with temperature, and during exceptionally warm years conch can reproduce year-round but with lower intensity (Randall 1964). At extreme temperatures, likely above the thermal threshold of conch, a reduction in reproductive behavior has also been observed (Appeldoorn et al. 2011). Although temperature and photoperiod are known

to influence the reproductive season, a generally supported linkage between any of these factors with the duration of the reproductive season has not been established. A closed season is a commonly applied management measure to protect spawning queen conch adults and should ideally overlap with the highest intensity in reproductive behavior for maximum effectiveness of the management strategy. Thus, detailed information on the reproductive season, and the ability to predict possible future changes in its duration and intensity, is important to maximize the protection of spawning conch when necessary.

1.4 Habitat preference and spatial distribution

Queen conch have a complex patchy distribution pattern, with large uninhabited areas between higher density patches (Vallès & Oxenford 2012, Kough et al. 2018). The species' habitat preferences and demands, which govern aspects of population dynamics, are poorly understood (Rosenzweig 1991, Kristan 2003, Gaillard et al. 2010). Queen conch is most common in depths < 25 m (Ehrhardt & Valle-Esquivel 2008), however, it can be found up to depths of 60 m (Randall 1964). The depth range of conch is believed to be restricted primarily based on light attenuation, which limits their photosynthetic food sources (e.g., filamentous alga) (Randall 1964, Ray & Stoner 1994, Creswell 1994). Queen conch are very sensitive to pollutants, increased turbidity, and sedimentation (Sanders 1984, Glazer & Berg 1994, Glazer & Quintero 1998). Adults prefer clear coarse sand/algal flats, although they can also be found on hard bottom habitats such as coral rubble (Torres-Rosado 1987, Stoner et al. 1994, Acosta 2001, Stoner & Davis 2010). Conch are, however, rarely found on soft bottoms (silt/mud) or in areas with high coral cover (Acosta 2006). Tidal channels with increased water flow between islands are also of ecological importance and have a positive association with conch of all age classes (Kough et al. 2018). Juvenile conch have more specific habitat requirements than adults (Stoner et al. 1994), and are primarily associated with native seagrass such as *Thalassia testudinum* and *Syringodium filiforme* across large parts of their distribution range (e.g. The Bahamas, USVI, Cuba, Turks & Caicos) (Hesse 1979, Alcolado 1976, Weil & Laughlin 1984, Stoner et al. 1994, Stoner 2003, Doerr & Hill 2013). These seagrass beds provide both nutrition and protection from predators (Ray & Stoner 1995, Stoner & Davis 2010). Juveniles can, however, also be found in coral-rubble and algae-covered hard bottom habitats (e.g. US. Virgin

Islands, Florida) (Randall 1964, Glazer & Berg 1994). Neither habitat, depth, nor sediment are, on their own, good indicators of the suitability of an area for conch, and the precise influences of these and other environmental variables (e.g. water flow, macrophyte cover) on the spatial distribution of queen conch are uncertain.

1.5 Population connectivity and genetics

Immigration (I) and emigration (E) in queen conch are mainly pertinent to the dispersal of larvae due to the relatively limited mobility of settled conch (Glazer et al. 2003, Delgado & Glazer 2007, Doerr & Hill 2013) preventing individuals to navigate over even minor elevations or steep slopes. Dispersal patterns are not well understood, and although they have a potential dispersal range of hundreds of kilometers (Appeldoorn 1994, Stoner & Davis 1997), other empirical observations indicate that this may be limited (Posada & Appeldoorn 1994, de Jesús-Navarrete & Aldana-Aranda 2000, Delgado et al. 2008). After eggs hatch, conch larvae are generally planktonic for 14-28 days (D'Asaro 1965) and disperse with surface currents (Appeldoorn 1994). Early genetic studies of queen conch using electrophoretic methods found a high degree of gene flow among populations dispersed over the species' geographic range, with definitive separation observed only between populations in Bermuda and those in the Caribbean basin (Mitton et al. 1989). Low genetic differentiation among locations at a local level was also found in the Mexican Caribbean with the use of mitochondrial DNA (Perez-Enriquez et al. 2011) and microsatellites (Zamora-Bustillo et al. 2011), as well in the Florida Keys and Bimini (The Bahamas) (Campton et al. 1992). However, during the last decade, advances in biophysical modeling and seascape genetics suggest that larval behavior of marine species, coupled with the complex hydrodynamics of the marine environment, may limit gene flow leading to fine-scale patterns of spatial genetic structure (Selkoe et al. 2010, D'Aloia et al. 2015, Iacchi et al. 2013, Thomas et al. 2015). A detailed understanding of the spatial genetic structure and the factors driving contemporary patterns of gene flow and genetic diversity are fundamental for understanding aspects of immigration and emigration in population dynamics. Furthermore, knowledge of the spatial genetic structure is necessary for conservation and management of marine fisheries by the identification of management units (i.e. populations with a low degree of connectivity requiring separate monitoring and

managing) (Palsbøll et al. 2007). Knowledge is also required to identify the appropriate spatial scale of marine protected areas (Gaines et al. 2010). Identification of management units and marine protected areas can span international boundaries and thus encourage efforts to conserve species whose ranges span international boundaries (Kough et al. 2013, Truelove et al. 2015).

1.6 Habitat degradation and pollution

The different life stages of queen conch (larvae, juvenile, adult) all have specific habitat requirements. However, the quality of each of these habitats and the quality of the water are important to queen conch in all life stages (Randall 1964, Stoner et al. 1992, Stoner 2003), and degradation of nearshore conch habitats has been linked to population declines (Posada et al. 1997). Due to the species' preference for breeding habitats with clean, coarse sand habitats with low organic content (Randall 1964, Stoner et al. 1992), these habitats can become unsuitable when subject to deposition of fine sediment or sediment with high organic content (Appeldoorn et al. 2011). Juvenile conch are particularly vulnerable to disturbances, since nurseries are mostly located in nearshore seagrass beds (Stoner 2003), which are often affected by land and sea-based human activities such as sedimentation, eutrophication, and boat activity. These can degrade seagrass beds and reduce available nursery habitats, as well as lower the quality of habitats, affecting survival and growth of juveniles (Orth et al. 2006). For example, the loss of seagrass in Barbados is likely to have contributed to low conch densities around the island (Vallès & Oxenford 2012), and in St Kitts and Nevis declines in the conch population has been linked to factors such as habitat dredging and hurricanes, which has decreased the quality of conch habitats (CITES 2012). Water quality also has an effect on larvae condition, and lowered oxidative reduction potential, often a consequence of eutrophication, has been observed to lower survival and increase duration of metamorphoses in larvae culture (Glazer & Quintero 1998). Pollutants (e.g. zinc, copper), and pesticides (i.e. tributyltin [TBT]), which are still sold and used in the Caribbean (Turner & Glegg 2014), have also been found to affect conch, causing for example reduced movement and feeding in juveniles (Sanders 1984). The decreased reproductive capabilities in adults found in some nearshore habitats in Florida is also believed to be influenced by pollutants such as zinc, which

has been found in the digestive tissue of nearshore conch and is known to cause reproductive deficiencies in gastropods (Laskowski & Hopkin 1996, Ducrot et al. 2007).

1.7 Invasive species

Another factor that has the potential to alter and possibly degrade high quality and already afflicted nearshore conch habitats is the invasion of alien species. The invasion of alien species has been recognized as an increasing global threat and is known, in many cases, to cause significant alterations in native populations, communities, and ecosystems over relatively short periods, as well as being linked to biodiversity loss (Clavero & Garcia-Berthou 2005, Kappel 2005, Venter et al. 2006). In the Caribbean, many non-native species have been introduced (Kairo et al. 2003) including *Halophila stipulacea* (Williams 2007), which is a seagrass species native to the Red Sea and the Indo-Pacific (Den Hartog 1970). Alterations to the composition of seagrass beds, such as through the introduction of invasive species, can cause changes in their associated biota and change the level of shelter and the quality and quantity of food sources (Willette & Ambrose 2012). *Halophila stipulacea* has, since its first observation on Grenada in 2002 (Ruiz & Ballantine 2004), spread rapidly and can now be found throughout the Eastern Caribbean and in the northern parts of South America (Vera et al. 2014, Willette et al. 2014). Juvenile conch occur in large parts of their distribution range (e.g. The Bahamas) primarily associated with native seagrass species, such as *Thalassia testudinum* (Stoner 2003), which provide both nutrition and protection from predators (Ray & Stoner 1995, Stoner 2003, Stoner & Davis 2010). Native seagrass detritus is an important component of the diet of juvenile conch, together with red and green macroalgae, primarily *Laurencia* spp. and *Batophora oerstedii* (Randall 1964, Stoner & Sandt 1991, Stoner & Waite 1991, Serviere-Zaragosa et al. 2009).

Halophila stipulacea has been shown to have a negative effect on native seagrass species *T. testudinum*, *S. filiforme*, and *Halodule wrightii* through displacement (Willette & Ambrose 2012, Scheibling et al. 2018). Knowledge of the impact of these seagrass changes on queen conch is limited, although studies have shown that changes in the associated fish assemblage and age structure can occur, with larger fish specimens being supported better in non-native

H. stipulacea beds (Willette & Ambrose 2012, Olinger et al. 2017). The rapid expansion of *H. stipulacea* in the Caribbean may have wide-ranging consequences for the structure and functioning of native seagrass ecosystems and the services they provide (Scheibling et al. 2018). The impacts of *H. stipulacea* on the quality of seagrass beds as nurseries, and how juvenile conch diet, growth, and survival are influenced is, to date, unknown. However, changes in habitat quality for juvenile conch could have severe consequences for population growth and the carrying capacity of conch populations around the Caribbean. Further information on the possible effects on conch populations found in areas where *H. stipulacea* is already present or will be present in the near future is required to adjust subsequent management and conservation strategies.

1.8 Thesis objectives and outline

The general objective of this study was to address knowledge gaps in the biology and ecology of queen conch so that the distribution and dynamics of the species in relation to its environment are better understood. Furthermore, practical methodological shortcomings in the surveying and assessment of queen conch were addressed. Additionally, a first insight into the possible effects of the rapidly expanding invasive seagrass *H. stipulacea* on conch in the Caribbean was investigated.

In **chapter 2**, the aim is to improve knowledge of queen conch reproductive biology (i.e. size-at-maturity and reproductive season). An evaluation and comparison of the relationship between shell lip thickness and maturity in queen conch throughout the Wider Caribbean Region, using histological analysis of queen conch gonads was carried out. A logistic model, commonly used to determine size at maturity for marine species, was compared with an accumulation model used in previous conch studies, which incorrectly over-estimates size at maturity in queen conch. Furthermore, the influence of seawater temperature on the length of the reproductive season was investigated. Posing the questions (1) does geographical variability in shell lip thickness at maturity follow the temperature-size rule? and (2) does the duration of the reproductive season, determined through behavioral observations, vary with latitude and temperature?

In **chapter 3**, the methodological shortcomings in the surveying of queen conch were addressed, and a novel towed video method was compared with a conventional survey method (i.e. belt transect using scuba divers) in a series of calibration transects in two different habitats (i.e. high complexity and low complexity). Posing the questions whether the underwater towed video method can (1) correctly identify live adult queen conch? and (2) accurately estimate adult queen conch densities so the method can be used as a survey tool capable of surveying queen conch throughout its entire depth range?

In **chapter 4**, the spatial distribution of adult queen conch and how it varies in response to a number of known abiotic and biotic variables between sites which vary in environmental conditions was examined. Posing the questions (1) is the patchy distribution and spatial dependency of conch observed by previous studies a general pattern in queen conch and what causes it? and (2) are significant densities of adult conch a common occurrence in deep areas (> 25 m) requiring a depth extension of conch surveys that are used to provide advice on fishing quotas?

In **chapter 5**, the diet and growth of juvenile conch in both native, mixed, and invasive seagrass beds using stable isotope analysis and *in situ* growth enclosure experiments were examined. Posing the questions (1) what are the most important food items in the different seagrass beds? and (2) is there a difference in juvenile conch growth between native and invasive seagrass beds which could potentially have an impact on population regrowth and carrying capacity in affected areas?

In **chapter 6**, the spatial genetic structure of the queen conch in the Caribbean using population genetic and multivariate analyses was examined. Posing the questions (1) is there evidence for population differentiation among localities within and between Caribbean countries? and (2) is spatial genetic structure randomly distributed in space, or is genetic structure among sites correlated with isolation by oceanic distance?

Finally, in **chapter 7** the findings of this study were summarized and discussed. The limitations of these findings were delineated, and suggestions for further research were proposed. The implications of these findings for management were also addressed, and lastly, the effects of

current and future challenges (e.g. climate change, habitat degradation, invasive seagrass) on queen conch population dynamics were discussed.

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Chapter 2

Variability in size at maturity and reproductive season of Queen Conch *Lobatus gigas* (Gastropoda: Strombidae) in the Wider Caribbean Region

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Fisheries Research 201: 18-25

Abstract

Queen conch (*Lobatus gigas*), is an economically and culturally important marine gastropod. The species is subject to extensive exploitation throughout large parts of the Caribbean which has led to a decrease in population densities across much of the species' distribution range. Hence, there is a need for protective measures to safeguard the reproductive stock. This requires a better estimation of its size at maturity, which is best quantified as the thickness of the lip that the shell develops after reaching its maximum length. The lip thickness at 50% maturity (LT_{50}) was determined using a logistic and an accumulation model, from seven representative locations of distribution of this species in the Wider Caribbean Region. LT_{50} of both females (7–14 mm) and males (4–11.5 mm) varied between different locations in the Caribbean, although it did not correspond with variation in water temperature. In most cases, females had a larger LT_{50} than males indicating sexual dimorphism. LT_{50} values estimated with the logistic model were smaller (7–14 mm for females, 4–11.5 mm for males) than values estimated with the accumulation model (13–26 mm for females, 16–24 mm for males), showing an overestimation of LT_{50} in queen conch in previous studies which used the accumulation model to estimate LT_{50} . Locations with a relatively high variation in water temperature had a significantly shorter reproductive season. The implementation of adequate minimum size regulation based on lip thickness (ca. 15 mm) and a Caribbean wide seasonal closure (May–September) using the most recent biological information from this study, taking into consideration the local differences in LT_{50} and reproductive season, will assist in developing a long-term sustainable queen conch fishery in the Caribbean.

2.1 Introduction

Lobatus gigas commonly called queen conch (Gastropoda: Strombidae), is an economically and culturally important marine gastropod (Brownell and Steveley, 1981; Appeldoorn 1994) found throughout the Wider Caribbean Region and the southern Gulf of Mexico. Queen conch has several biological characteristics (e.g. slow-moving, often occurring at shallow depths, and aggregating during the reproductive season), which makes it vulnerable to overfishing (Appeldoorn et al., 2011). The species is heavily exploited throughout large parts of its natural

range (Stoner, 1997; Acosta, 2002) and the overall harvest of queen conch has increased over the last 30 years in response to increased demand from the international market (Theile, 2005). Consequently, the increased harvest has led to a decrease in population densities and a truncation of size classes (Stoner et al., 2012c) across much of the species' distribution range (Acosta, 2006). Due to concerns for the continued overexploitation of the species, measures have been taken such as the listing of the species to Appendix II of the CITES in 1992 and the establishment of the International Queen Conch Initiative, which aims at improving regional management through harmonized regulations (Appeldoorn et al., 2011). Many Caribbean nations have taken measures with regards to queen conch protection and exploitation, but these are still mostly not harmonized among nations and recovery of overfished populations has been slow (Theile, 2001). An important biological reference for queen conch management regulations is the size at which 50% of the adult population is mature (L_{50}) (Roa et al., 1999), which aims at protecting juveniles and allowing the target organism to spawn at least once before caught, thereby ensuring the sustainability of the stock (Vasilakopoulos et al., 2011). In the Wider Caribbean Region queen conch reach sexual maturity at ca. 3.5–4 years old (Appeldoorn, 1988; Stoner et al., 2012a). After reaching its maximum length, the edge of the shell flares outward and develops a lip which increases in thickness prior to and also after the onset of maturity (Appeldoorn, 1988; Stoner et al., 2012a). Length growth, therefore, stops before maturity and thus shell length is not a reliable indicator of maturity. Lip thickness is a more appropriate index of maturity than shell length (Stoner et al., 2012a; Foley and Takahashi, 2017). Growth in lip thickness varies between different locations and has been found to be dependent on sex, depth, latitude, temperature, food availability, age class and shelter (Appeldoorn, 1988; Stoner and Sandt, 1992; Appeldoorn and Baker, 2013). Species with an extended distribution range often exhibit differences in size at maturity along temperature gradients (Brown-Peterson and Thomas, 1988; Irie et al., 2013), with higher developing temperatures causing early maturation at a smaller size, a plastic response called the temperature-size rule (Atkinson, 1994). The temperature-size rule has been observed in marine gastropods (Irie et al., 2013) but no evidence of this has been investigated for queen conch so far. However, given the relationship between lip thickness and maturity in queen conch and given that local conditions affect the growth rate of lip thickness with indications of higher growth rates in the center of the Caribbean region than the northern region

(Appeldoorn, 1988; Stoner and Sandt, 1992), local relationships between lip thickness and size-at-maturity may vary along temperature gradients according to temperature-size rule. Moreover, the reproductive season of queen conch also differs between locations in duration and intensity (extended reproductive season vs. discrete reproductive season with one or more peaks) across the Caribbean region (Avila-Poveda and Baqueiro-Cardenas, 2009; Aldana-Aranda et al., 2014). Therefore, knowledge of the relationships between lip thickness and maturity and an understanding of reproductive seasons are critical for the implementation of appropriate site-specific regulations to protect the spawning biomass (Foley and Takahashi, 2017). Most nations in the Caribbean region have implemented minimum harvest size regulations for queen conch (Theile, 2005), which are often based on outdated, limited biological information and an inappropriate indicator for maturity such as shell length (Stoner et al., 2012a). To improve the biological knowledge base on queen conch reproduction and hence to contribute to more rational management measures, this study aims to (1) evaluate and compare, the relationship between shell lip thickness and maturity in queen conch throughout the Wider Caribbean Region using histological analysis of queen conch gonads; (2) to compare a logistic model, commonly used to determine size at maturity (L_{50}) for marine species, with an accumulation model (Avila-Poveda and Baqueiro-Cardenas, 2006) used in previous studies to determine size at maturity (L_{50}) in queen conch, but not used for this purpose for other species; (3) to test the hypotheses that geographical variability in shell lip thickness at maturity will follow the temperature-size rule and that the duration of the reproductive season, determined through behavioral observations will vary with latitude.

2.2 Material and methods

2.2.1 Queen conch collection and study sites

Collection of female and male flared-lip queen conch for size-at-maturity estimates determined through histological analysis was realized on a rubble, sand, algae and/or seagrass bottom at depths between 2–30 m, between 2003 and 2016 at seven locations covering the Wider Caribbean Region (Figure 2.1): (1) in The Bahamas [Abaco (26.1°N, 77.5°W), $n = 57$ conch during June 2012 and Exuma Cays (24.2°N, 76.4°W), $n = 102$ samples June-July 2012];

(2) in St Barthélemy (17.9°N, 62.9°W), n = 63 conch during August 2016; (3) in St Eustatius (17.5°N, 63.0°W), n = 83 conch during May-August 2013 and July- August 2015; (4) on Saba Bank (17.4°N, 63.3°W), n = 83 conch during July 2014 and August 2015; (5) in Barbados (13.0°N, 59.4°W), n = 65 conch during July-September 2009 and June-September 2010; (6) in San Andres Archipelago, Colombia (12.3°N, 81.4°W), n = 202 conch from February 2003 to January 2004. Queen conch were collected from nine lip thickness intervals between 0 and 45 mm. Although effort was made to sample a minimum of five conch from each of the lip thickness intervals of both genders at each location, due to the difficulty of finding males with < 10 mm lip thickness on Saba Bank, only 1 conch from the < 5 mm lip thickness interval and two conch between 5 and 9 mm lip thickness interval were sampled at this location. Gonads for histological analysis were sampled during the peak reproductive season for each location when the frequency of reproductively active conch were at its peak to ensure that the conch collected had fully developed gonads. Reproductive seasonality in queen conch was estimated through behavioral observations on seagrass and algae bottom at depths of 9–25 m in the Saba national marine park (17.6°N, –63.3°W) during September 2013 until May 2015 and in the southern marine reserve (no-take zone) in the St Eustatius national marine park (17.5°N, –62.9°W) during May 2013 until April 2014 (Figure 2.1).



Figure 2.1. Location of the sampling areas (circles) in the Wider Caribbean Region for the evaluation of size at maturity and reproductive season of queen conch (*Lobatus gigas*).

2.2.2 Histology

Queen conch collected for size-at-maturity estimates were first removed from their shell and sex was determined by visually examining of the conch noting the presence of a verge (male) or an egg grove (female). Afterward, the conch were dissected and the visceral mass (that includes gonad and digestive gland) were extracted. A 1 cm³ sample of the gonad tissue was removed for histological preparation according to Avila-Poveda and Baqueiro-Cardenas (2009). Each tissue sample was fixed in 10% neutral buffered formalin during seven days. Next, the samples were gradually dehydrated in a series of ethanol solutions (70%, 90%, 96%), cleared in a 1:1 mixture ethanol-historesin® and finally infiltrated and embedded in historesin®. Serial sections were cut with a rotary microtome (4 µm thick) and mounted on glass slides, which were stained with Hematoxylin and Eosin.

2.2.3 Determination of maturity

Samples were assigned a classification of non-mature and mature based on microscopic examination of the histological sections made from the gonads following a maturity scale. Samples from St Barthélemy, St Eustatius, and Saba Bank, were classified following a maturity scale (Table S2.1, stage 0–2 = non-mature, stage 3–6 = mature), adapted from Delgado et al. (2004). Samples from the two locations in The Bahamas (Abaco, Exuma Cays) were classified following procedures also adapted from Delgado et al. (2004) and samples from Barbados and San Andrés Archipelago (Colombia) were classified following procedures adapted from Avila-Poveda, (2004). Although these scales have different number of classification stages, the classification of non-mature vs mature conch is identical, as it is based on the presence or absence of the same structures (Avila-Poveda, 2004; Stoner, 2012) and therefore the maturity data of all samples can be directly compared (Avila-Poveda and Baqueiro-Cardenas, 2009; Stoner et al., 2012a, Table S2.1).

2.2.4 Size at maturity

Size at maturity was defined as the lip thickness at which 50% of the population (LT_{50}) of females and males separately were sexually mature. To determine LT_{50} and the relationship between shell lip thickness and maturity, a binominal (0 = non-mature, 1 = mature) logistic model was used (Roa et al., 1999):

$$P_{LT} = \frac{1}{1 + e^{(\beta_1 + \beta_0 \cdot LT)}}$$

where P_{LT} = proportion mature of each lip thickness class, β_0 = intercept, β_1 = slope, and LT = Lip thickness (mm). $LT_{50\%}$ and its 95% confidence limits are then estimated as:

$$\widehat{LT}_{50\%} = \frac{-\hat{\beta}_0}{\hat{\beta}_1} \pm z_{0.025} \cdot \hat{\sigma}_{LT_{50\%}}$$

where $z_{0.025}$ is a quantile from the normal distribution. An accumulation model used in previous studies (Bissada, 2011; Stoner et al., 2012a) was also applied to the same samples and compared to the LT_{50} values of the logistic model. This model plots the accumulated number of mature individuals for each lip thickness grouped interval (Avila-Poveda and Baqueiro-Cardenas, 2006).

2.2.5 Reproductive season

The reproductive season was quantified by calculating the percentage of flared-lip queen conch (females and males) with a well-developed lip ≥ 10 mm (Appeldoorn, 1988) participating in confirmed reproductive behaviors (copulation and egg-laying) for each month during a calendar year. During monthly surveys, two to three scuba divers searched the study areas (Saba, St Eustatius) for queen conch ($n = 100\text{--}300$ per month) and classified each conch encountered to a reproductive group as described by Stoner et al. (1992); (1) Pairing: two conch were aligned, with the anterior part of the shell of one animal overlapping the posterior part of the shell of the other; but copulation was not observed. (2) Copulating: animals were engaged in copulation, with the verge of the male beneath the mantle of the female. (3) Egg-laying: a female was actively laying an egg-mass. (4) Non-reproductive: conch was not engaged in reproductive behavior. (5) Egg-mass: presences of solitary egg-mass. On St Eustatius, all months within the survey period (May 2013 until April 2014) were used to define the reproductive season. On Saba, surveys from the entire survey period (September 2013 until May 2015) were combined to define the reproductive season for a full calendar year. Data for each calendar month during this period with the highest frequency of reproductive behaviors were used to avoid months with very low densities of flared-lip conch (May-August 2013, February-May and September 2014).

Table 2.1. Overview of spatial variation in the reproductive season for queen conch (*Lobatus gigas*) in the Wider Caribbean Region based on observed reproductive behavior (copulation, egg-laying, presence of solitary egg-mass) for a full calendar year. Colors indicate the relative activity of observed reproductive behaviors (black = high activity of reproductive behaviors, grey = medium activity of reproductive behaviors, light grey = low frequency of reproductive behaviors ($< 1\%$), white = no reproductive behaviors observed).

Location	Months												Months	Lat	Long	Reference
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec				
Florida													4.5	25.7	-80.1	D'Asaro, 1965
The Bahamas													4.5	23.5	-77.8	Stoner et al., 1992
Mexico													8	22.3	-89.7	Perez & Aranda, 2002
Turks & Caicos													6	21.9	-72.1	Davis et al., 1984
St John (USVI)													8	18.3	-64.7	Randall, 1964
St Croix													9	17.8	-64.8	Coulston et al., 1987
St Eustatius													9	17.5	-63.0	This study
Barbados													8	13.2	-59.4	Bissada, 2011
Venezuela													7.5	11.8	-66.7	Weil & Laughlin, 1984

Table 2.2. Shell lip thickness (LT) parameters observed for maturity in queen conch in different parts of the Caribbean region with sample size (n). LT₅₀: LT at which 50% of the population was sexually mature for females (A) and males (B). Temp: annual mean ocean temperature (°C). Var: variation in annual ocean temperature (°C). Minimum size regulation for the corresponding locations (NR = no minimum size regulation in place, LT = minimum size regulation based on lip thickness, SL = minimum size regulation based on shell length, Lip = minimum size regulation requires conch to have formed a lip).

A) Female		Logistic regression model		Accumulation model							
Location	n	LT ₅₀ F (mm)	95% CI	LT ₅₀ F (mm)	LTmin	Temp (°C)	Var (°C)	Lat	Long	Min size regulation	
Abaco (The Bahamas)	30	13	10-15	17	11	26.1	4.5	26.1	-77.5	NR	
Exuma Cays (The Bahamas)	51	14	10-17	26	12	26.6	3.8	24.2	-76.4	NR	
St Barthélemy	32	9.5	6-13	23	8	27.9	0.6	17.9	-62.9	LT 7 mm	
St Eustatius	45	11	5-16	25.5	9	27.8	0.6	17.5	-63.0	SL 190 mm	
Saba Bank	45	7	4-10	25	8	27.9	0.7	17.4	-63.3	Lip	
Barbados	25	10	2-16	18.8	2	28.0	1.4	13.0	-59.4	NR	
San Andrés Archipelago (Colombia)	98	8	1-13	13	3	28.2	1.1	12.3	-81.4	NR	

B) Male		Logistic regression model		Accumulation model							
Location	n	LT ₅₀ M (mm)	95% CI	LT ₅₀ M (mm)	LTmin	Temp (°C)	Var (°C)	Lat	Long	Min size regulation	
Abaco (The Bahamas)	27	8	5-10	16	8	26.1	4.5	26.1	-77.5	NR	
Exuma Cays (The Bahamas)	51	11	7-14	24	9	26.6	3.8	24.2	-76.4	NR	
St Barthélemy	31	11.5	8.5-15	19.5	9	27.9	0.6	17.9	-62.9	LT 7 mm	
St Eustatius	38	4	0-9	21	6	27.8	0.6	17.5	-63.0	SL 190 mm	
Saba Bank	38	9	4-12	19	9	27.9	0.7	17.4	-63.3	Lip	
Barbados	40	5	0-10	19.2	5	28.0	1.4	13.0	-59.4	NR	
San Andrés Archipelago (Colombia)	124	4	0-8	17.5	3	28.2	1.1	12.3	-81.4	NR	

2.2.6 Statistical analysis

Size at maturity LT₅₀ values from the logistic model were analyzed for females and males separately as well as the minimum lip thickness at maturity (LT_{min}) found at each location. Data from male conch from Saba Bank were excluded from the analysis, because of the low sample size of the lower lip thickness sizes (n = 3, < LT 10 mm) causing the predicted LT₅₀ and LT_{min} values to become unreliable. LT₅₀ and LT_{min} values were related (separately for males and females) to three potential explanatory environmental variables: latitude, annual mean temperature, and variation in annual mean temperature, using the nonparametric Kendall rank correlation, which is preferred over other nonparametric association tests in the case of

small sample sizes (Gibbons and Chakraborti, 2011). Temperature data for all locations were obtained from the World Ocean Atlas database (WOA, 2013a, 2013b) and calculated from mean monthly temperatures at a depth of 15 m from 1994 to 2012. Where temperature data were not available for the precise location, the closest location where temperature data were available was used. For the five locations (Abaco, St Barthélemy, Saba Bank, Barbados, San Andrés Archipelago) for which the closest proxy location available was used, the deviation in latitude ranging from 0.1–0.3 decimal degrees from the location where queen conch were collected. A Wilcoxon signed rank test was used to test whether LT_{50} in females was larger than in males, as is common in many species of gastropods (Bigatti et al., 2008; Cledón et al., 2008). A Kendall's rank correlation was calculated between LT_{min} and LT_{50} to investigate a possible relationship between LT_{min} and LT_{50} for females and males separately and to evaluate the use of LT_{min} as a predictor of LT_{50} . Finally, a paired Wilcoxon signed rank test was used to test the difference between the LT_{50} values of the logistic model and the corresponding values of the accumulation model used in previous studies (Avila-Poveda and Baqueiro- Cardenas, 2006; Stoner et al., 2012a). In all cases, nonparametric tests were performed given the non-normal distribution of residuals. Data analysis was performed using the software environment R (R Development Core Team, 2016).

2.2.7 Reproductive season

The relationship between mean annual temperature, variation in annual temperature and duration of reproductive season was explored using data from this study (St Eustatius) and from previously reported studies from eight other locations: Florida (25.7°N, -80.1°W) (D'Asaro, 1965), The Bahamas (23.5°N, -77.8°W) (Stoner et al., 1992), Mexico (22.3°N, -89.7°W) (Perez-Perez and Aldana-Aranda, 2002), Turks and Caicos (21.9°N, -72.1°W) (Davis et al., 1984), St John, USVI (18.3°N, -64.7°W) (Randall, 1964), St Croix, USVI (17.8°N, -64.8°W) (Coulston et al., 1987), Barbados (13.2°N, -59.4°W) (Bissada, 2011), Venezuela (11.8°N, -66.7°W) (Weil and Laughlin, 1984)(Table 2.2). Reproductive seasonality data from these locations (Table 2.1) have been evaluated in a comparable way through behavioral observations of confirmed reproductive behaviors (i.e. copulation, egg-laying, the presence of egg-mass) during a full calendar year using monthly, or more frequent, dive or snorkel surveys. Also, in this case, temperature data were obtained from the World Ocean Atlas database

(WOA, 2013a, 2013b). For the six locations (Barbados, St John, St Croix, Florida, Mexico, Turks and Caicos, Venezuela) which used a proxy location, the deviation in latitude ranged from 0.2–0.4 decimal degrees from the location where the behavioral study was conducted. Kendall's rank correlation was calculated between the duration of the reproductive season and temperature, as residuals were not normally and/or heterogeneously distributed.

2.3 Results

2.3.1 Lip thickness at maturity

Lip thickness at first (onset) maturity (LT_{min}) ranged between 2 mm (Barbados) and 12 mm (Exuma Cays) for females and between 3 mm (San Andrés Archipelago) and 9 mm (Exuma Cays, St Barthélemy, Saba Bank) for males across the study area (Table 2.2). LT_{50} ranged between 7 mm (Saba Bank) and 14 mm (Exuma Cays) for females and between 4 mm (San Andrés Archipelago) and 11.5 mm (St Barthélemy) for males across the study areas (Table 2.2). Females had a significantly larger LT_{50} than males (one-sided Wilcoxon signed-rank test: $n = 6$, $T = 20$, $p = 0.03$) in all locations except St Barthélemy. A significant relationship was also found between LT_{min} and LT_{50} for females ($n = 7$, $\tau = 0.68$, $p = 0.03$) but not for males ($n = 6$, $\tau = 0.64$, $p = 0.08$). The LT_{50} values derived from the logistic model were always lower than those derived from the accumulation model for both females ($n = 7$, $T = 0$, $p = 0.02$) and males ($n = 6$, $T = 0$, $p = 0.03$) (Table 2.2). No relationship was found between any of the potential drivers of geographical variability tested (latitude, mean annual ocean temperature, and variation in annual ocean temperature) and LT_{50} for females ($n = 7$, $\tau = 0.53$, $p = 0.14$; $n = 7$, $\tau = -0.51$, $p = 0.12$; $n = 7$, $\tau = 0.59$, $p = 0.07$, respectively) or males ($n = 6$, $\tau = 0.41$, $p = 0.25$; $n = 6$, $\tau = -0.36$, $p = 0.33$; $n = 6$, $\tau = 0.14$, $p = 0.70$, respectively).

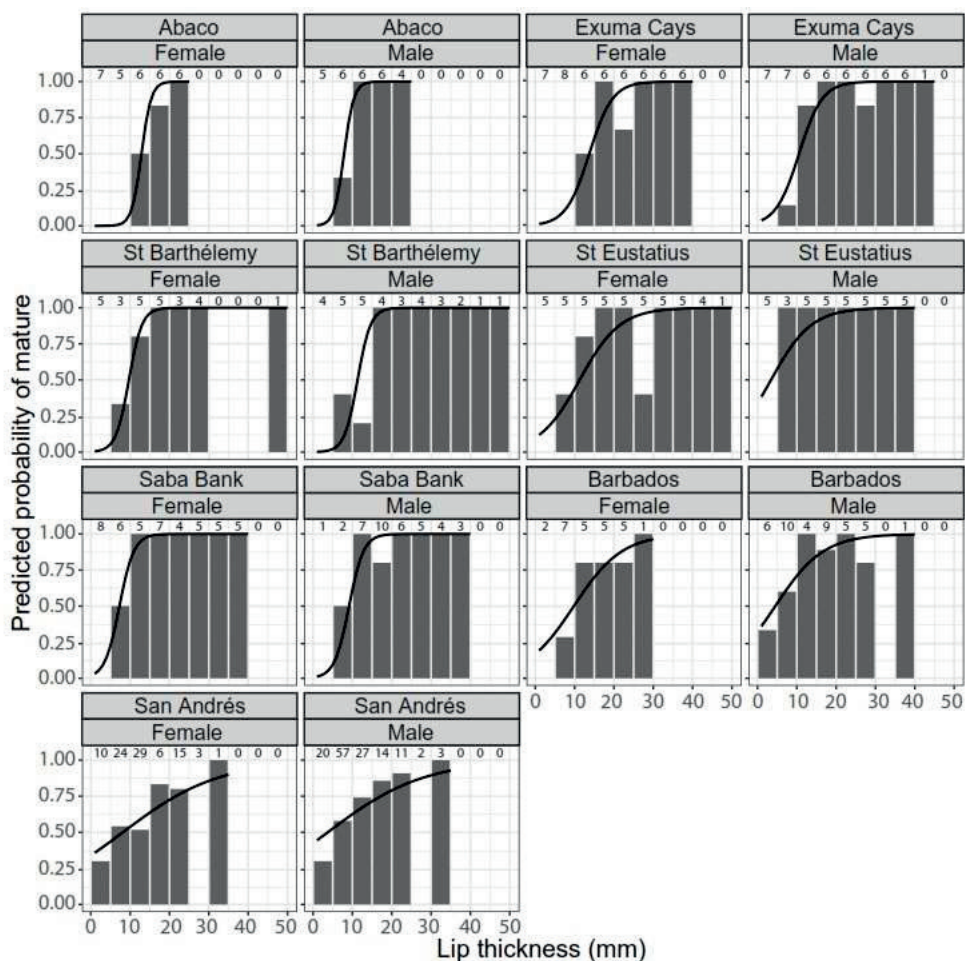


Figure 2.3. Logistic curve (black line) of relative frequency as a function of lip thickness size of queen conch (*Lobatus gigas*) for females and males separately. Bars show the percentage of mature individuals in each size interval with sample size of the size interval above bars.

2.3.2 Reproductive season and temperature

Reproduction of queen conch differed between St Eustatius and Saba in the duration of the reproductive season and in the frequency of reproductive behavior (percent of the population engaged in egg-laying or copulation) (Table 2.3, S2.4). The reproductive season in St Eustatius began towards the end of February and continued until November during which < 1–26.5% of all flared-lip conch encountered were engaged in reproductive behavior (Table 2.3). The reproductive season on Saba began in June and ended in September during which 2.5 – 24%

of all conch encountered were engaged in reproductive behavior (Table S2.4). A peak in reproduction was observed between May and September in St Eustatius and in July and September in Saba during which between 15.4-26.5% and 7.9-24%, of all flared-lip conch were engage in reproductive behaviors respectively. A significant relationship was found between variations in annual water temperature and duration of the reproductive season, where locations with a high variation in water temperature had a shorter reproductive season than locations with a low variation in water temperature ($n = 9$, $\tau = -0.75$, $p < 0.01$) (Figure 2.2). Mean annual temperature ($n = 9$, $\tau = 0.51$, $p = 0.07$) and latitude ($n = 9$, $\tau = -0.49$, $p = 0.11$) did not show a significant relationship with the duration of the reproductive season.

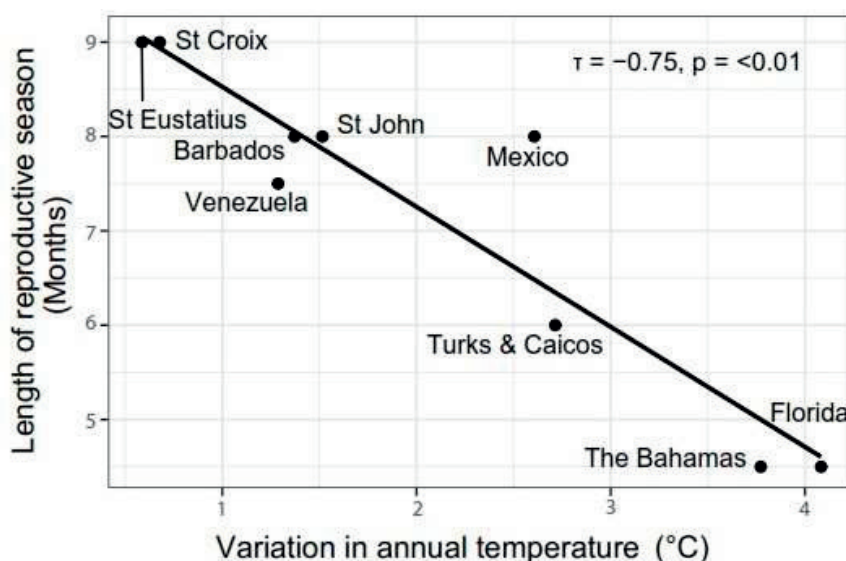


Figure 2.2. Relationship between variation in annual mean ocean temperatures and duration of queen conch (*Lobatus gigas*) reproductive season (data on reproductive seasonality from Table 2.1).

2.4 Discussion

2.4.1 Size at maturity

There is a clear positive relationship between the thickness of the shell lip and the onset of maturity in queen conch. Although this relationship showed some variation between different locations in the Caribbean (Figure 2.3, Table 2.2) it did not correspond with variation in water

temperature and did not follow the temperature-size rule, which was hypothesized. Annual variation and mean annual temperature differences between the tropical Caribbean region ($< 23^{\circ}\text{N}$) and the subtropical Caribbean region ($> 23^{\circ}\text{N}$) (northern Bahamas) may not be large enough to noticeably affect size at maturity in queen conch in terms of differences in LT_{50} with temperature. In almost all cases females had a larger lip thickness size at 50% maturity and a significant difference was found in LT_{50} between the sexes. Foley and Takahashi (2017) evaluated LT_{50} for queen conch in Belize using the same logistic model as in this study and also found a larger LT_{50} for females than males. The results from this and the previous study (Foley and Takahashi, 2017) indicated that there is a degree of sexual dimorphism in LT_{50} in queen conch. Sexual dimorphism, with females being larger than males at maturity, is common in many species of gastropods (Gharsallah et al., 2010; Riascos and Guzman, 2010). The difference in shell lip thickness at 50% maturity between the sexes might be explained by the hypothesis of sexual selection, where increase in size corresponds with increased fecundity for females (Shine, 1989; Ilano et al., 2004). There is evidence for this in some gastropods (Erlandsson and Johannesson, 1994; Cruz et al., 2001), and gonad weight increases in female queen conch up to 23 mm LT, suggesting increased fecundity with increased lip thickness (Stoner et al., 2012a). However, further studies would be necessary to confirm if this hypothesis can also explain sexual dimorphism in LT_{50} for queen conch. Also, new studies would be needed to determine whether females reach size at 50% maturity at a similar age as males due to faster growth rates in females or if growth rates between the sexes are similar and thus females mature at an older age than males. Lip thickness at first maturity (LT_{\min}) gives an indication of at what lip thickness conch begin to mature. However, the fact that LT_{\min} is based on only one observation and is strongly dependent on the total number of observations from conch in the lower lip thickness range limits the value of LT_{\min} as an indicator of the onset of maturity in a population. Lip thickness at 50% maturity (LT_{50}), however, is an estimate based on the whole population and thus a much more reliable measurement of size at maturity. The confidence intervals around LT_{50} suggest that in some locations (St Eustatius, San Andrés Archipelago, Barbados) maturity in male conch could occur before the development of a lip (Table 2.2). However, given behavioral observations from this and previous studies throughout the Caribbean region, which have not observed conch without a lip engaging in any reproductive behaviors (copulation, egg-laying), this is unlikely. The diffuse reproductive

season in the San Andrés Archipelago, with a low number of reproductively active conch throughout the year, and a reproductive season with several peaks in Barbados (Aldana-Aranda et al., 2014) could have caused the shallow regression curves for these locations, as mature conch would appear histologically non-mature when sampled during periods between spawning cycles. This is apparent from the higher percentage of conch with relatively thick lips (> 15 mm) appearing histologically non-mature in Barbados and the San Andrés Archipelago compared to the other locations (Figure 2.2). In the case of St Eustatius, the logistic regression was unreliable because of the low number of conch in the deflection point (0–10 mm size class) and because of the complete separation with 100% non-mature individuals < 5 mm lip thickness and 100% mature individual ≥ 5 mm lip thickness (Figure 2.3). The LT_{50} values derived from the logistic model in this study were consistently lower than the respective LT_{50} values derived from the accumulation model used by previous studies (Avila-Poveda and Baqueiro-Cardenas, 2006; Bissada, 2011; Stoner et al., 2012a). The main cause of this is that the accumulation model only takes mature individuals into account when deriving the maturity curve as well as LT_{50} . Moreover, it does not provide confidence intervals around the LT_{50} estimates unless repetitive sampling is conducted, which was never reported in previous studies. In addition, the order in which mature individuals are added to the total, influences the shape of the curve (Colwell and Coddinton, 1994). Causing it to become very sensitive to disproportional sampling and thus is easily skewed in either direction if sampling is not completely proportionate. The logistic model on the other hand takes into account all sampled individuals (mature and non-mature) and the deflection point (with 95% confidence interval) where 50% of the population is mature (LT_{50}) is less sensitive to disproportional sampling, although sufficient observations during the reproductive season are needed to prevent confidence limits that are too wide, such as in the case of male conch in St Eustatius, San Andrés Archipelago, and Barbados. The direct comparisons of the logistic and accumulation models in this study show that the accumulation model very likely overestimated LT_{50} in queen conch in previous studies due to the characteristics of the model. Therefore, we recommend that all future studies apply the logistic model for determination of LT_{50} .

2.4.2 Reproductive season

Despite their close proximity (ca. 30 km), the reproductive season for queen conch around St Eustatius was longer (ca. 9 months) and had a higher percentage of flared-lip conch engaging in reproductive behavior (< 1–26.5%) than near Saba (ca. 4 months, 2.5–24%). However, the shorter season and lower reproductive activity on Saba were most likely caused by fishing activity on the small study site, which severely lowered the flared-lip conch density during several months of the year. Densities during some months dropped below the threshold (60 flared-lip conch/ha) for successful reproduction to occur (Stoner and Ray-Culp, 2000). Thus, the true extent of the reproductive season on Saba was most likely masked due to low densities of flared-lip conch in the survey area. Variation in annual ocean temperatures was found to have a significant negative effect on the duration of the reproductive season and appears to be a more important factor than mean annual temperature. Indications of this have previously been seen as fluctuations in sea temperature have been reported to initiate and stop reproductive behavior in queen conch (Brownell, 1977; Stoner et al., 1992). However, Stoner et al. (1992) did indicate that photoperiod is also important and might be even more important than temperature in regulating the duration of the reproductive season in queen conch. Tuning of reproductive strategies (intensity of reproductive behaviors and duration of the reproductive season) in response to a variable temperature is common among marine gastropods (Lewis, 1986; Ribeiro et al., 2009), other benthic invertebrates (Bauer, 1992; Aragón-Noriega and Alcántara-Razo, 2005) and fish (Brown-Peterson and Thomas, 1988; Snyder and Peterson, 1999), and is formulated as the classic paradigm of continuous reproduction in low latitude areas and more restricted reproduction with an increase in latitude (Bauer, 1992; Van De Kerk et al., 2016). The effects of variations in annual temperature on the duration of the reproductive season in queen conch have previously been postulated by Aldana-Aranda et al. (2014), who found some evidence for this relationship. They further suggested that reproductive strategy (intensity of reproductive behaviors and duration of the reproductive season) may have a genetic component. However, further studies will be required to more accurately understand detailed connectivity patterns of queen conch (Truelove et al., 2017) and whether differences in reproductive strategies are caused by adaptation to local conditions or by phenotypic plasticity.

2.4.3 Implications for fisheries management

The majority of Caribbean nations have implemented regulations regarding minimum shell length, ranging from 178 to 229 mm (Table S2.2), although this has been demonstrated to be a poor indicator for sexual maturity in queen conch (Stoner et al., 2012a). Another common regulation is the requirement for a flared lip (e.g., The Bahamas), however, this does not guarantee that the landed conch will be sexually mature as first onset of maturity occurs only after formation of the lip (Stoner et al., 2012a; Foley and Takahashi, 2017; this study). The majority of nations have not implemented minimum size regulation with regards to lip thickness, which was also the case for all sampling areas in this study, except for St Barthélemy (Table 2.2, S2.2). Only a few nations (i.e. Antigua and Barbuda, Cuba, Nicaragua, Puerto Rico, St Barthélemy, and US Virgin Islands) have specified regulations regarding lip thickness, ranging from 5 to 9.5 mm lip thickness (Table S2.2), which in most are in the lower confidence interval range for the LT_{50} values found in this study. Thus, the vast majority of nations in the Caribbean region have regulation in place which is not sufficient to ensure that at least 50% of the queen conch population will reach maturity before being harvested. However, with a growing body of evidence suggesting the efficacy of conserving reproductive stocks based upon shell thickness criteria it is likely that more nations will implement thickness-based regulations for queen conch harvest. Our results show that previous estimates of LT_{50} using an accumulation model have overestimated size at maturity for queen conch. Furthermore, our results also suggest that some geographical variation in LT_{50} exists, although this variation could not be tied to a specific factor. The results of LT_{50} presented in this study using a logistic model should be considered when setting minimum size regulations for the studied areas. Queen conch in areas outside the range of the locations in this study could have different LT_{50} values and therefore it is recommended to further investigate local conditions of size (lip thickness) at maturity. A closed season is a commonly applied measure to protect spawning adults, although this does not always overlap with the peak reproductive season of conch in respective areas (Table 2.1, S2.3, S3.4). Currently, 11 of 33 locations (Table S2.3), have a closed season between June and August, and only five of 33 locations have a closed season from May through September, excluding locations with a year-round closure (Table S2.3). Notably, one large exporter of queen conch, The Bahamas, has no closed season. Even though the timing may vary slightly between years (Appeldoorn and Baker, 2013) and locations (Table S2.4), the

reproductive season of queen conch occurs simultaneously in the vast majority of locations in the Wider Caribbean Region between May and September (Table 2.1, S2.4). This provides an option for regional coordinated management regulations; Caribbean-wide closure from May through September, which would maximize the protection of spawning flared-lip conch (mature adults) in the largest amount of locations and improve compliance generally. Regional coordination of a closed season for queen conch fishing has been routinely discussed and promoted at multinational fishery management meetings during the last several years. Progress is being made, and we are optimistic that the regional analysis presented here will help to accelerate the implementation of badly needed management strategies. The effects of current queen conch fishing practices have in many locations resulted in declining stocks (Stoner and Schwarte, 1994) and truncation of age and size structure (Stoner et al., 2012b, c). This and previous studies show that the current minimum size and closed season regulations in the Wider Caribbean Region should be adapted according to the newest insights in the biology of queen conch (Stoner et al., 2012a; Aldana-Aranda et al., 2014). Implementation of adequate minimum size regulation based on lip thickness (ca. 15 mm) and a Caribbean-wide seasonal closure (May – September) using the most recent biological information from this study, taking into consideration the local differences in LT_{50} and reproductive season, will be an important step in the right direction to improve management of the species. These can together with other measures, such as recommended by the Queen Conch Expert Workshop Group Report (2012) assist in developing a long-term sustainable queen conch fishery in the Caribbean.

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Supplementary material

Table S2.1. Reproductive phases and classifications scheme for describing queen conch maturity (nonmature = stage 0-2, mature = stage 3-6) used in this study for females (F) and males (M) (adapted from Delgado et al., (2004) and developed by Wageningen Marine Research).

Gonad developmental phase	Stage	Definition
Undifferentiated (Non-mature)	0	No germ tissue or undifferentiated; almost non to no Gonadal tissue development. This is an abnormal condition in adult females and males. Gonad composed out of 90-100% of connective tissue. Few developing follicles can be detected. There are no gametes. It is not possible to determine sex histologically. Some phagocytes can be detected and maybe a random primary oocyte.
Primary Development (Non-mature)	1	Some germ tissue present in the shape of mainly primary oocytes (F) or spermatogonia (M). Some phagocytes can be detected. Gonad composed out of > 75% connective tissue.
Early Development (Non-mature)	2	Germ tissue present in the shape of primary oocytes and cortical alveolar oocytes (F) or spermatogonia and spermatocytes. Germ tissue covers > 25% of the gonadal area.
Mid Development (Mature)	3	Germ tissue in females will mainly be present in the shape of early vitellogenic oocytes, they may be grouped in four to five cells Early vitellogenic oocytes and earlier shapes can all be seen in these perfect little nests. It is also possible that we can see some oviducts but they will be empty. The males may show all stages of spermatogenesis and it is possible that (the beginnings of) a vas deference is present, but it will be without spermatozoa in it.
Late Development (Mature)	4	Germ tissue in females will mainly be present in the shape of late vitellogenic oocytes, the nests start to merge together and within these nests all stages of oocytes may be present. Oviducts will most likely be present but mainly don't contain any oocytes. The males will show all stages of spermatogenesis and there will be empty or columnar vas deference present (there may be some atypical spermatozoa in the vas deference but no large quantities).
Ripe (Mature)	5	This will show animals that are spawning. The females will show all signs from stage 4 plus that there will be oocytes within the oviducts, you will also see follicles that are dispersed and with holes in their walls. There can also be some (almost) empty follicles present. The males also show similarity to stage 4 with the difference shown in the full and swollen vas deference also here you can find some dispersed, with holes in their walls and even (almost) empty follicles.
Spent (Mature)	6	This is the post-spawning phase. You will mainly (> 50%) see (almost) emptied follicles, reabsorption of late vitellogenic oocytes (F) or deteriorating and stretched follicles and a vas deference which is reduced in size and empty (M). There might also be Atresia and phagocytes.

Table S2.2. Reproductive phases and classifications scheme for describing queen conch maturity (non-mature = stage 0-2, mature = stage 3-6) used in this study for females (F) and males (M) (adapted from Delgado et al., (2004) and developed by Wageningen Marine Research).

Location	Minimum SL (mm)	Minimum LT (mm)	Lip	Minimum Weight (g)	Relevant Legislation
Anguilla	180	NR	NR	225 D	Revised Regulations of Anguilla under Fisheries Protections Act R.S.A c. F40
Antigua & Barbuda	180	5	Yes	225 D	Fisheries Act, No.14 of 1983 and the Fisheries Regulations, No. 2 of 2013
Aruba	PCS	PCS	PCS	PCS	Marine Environment Ordinance, Article 5, 1980. Amendment Article 5, No. 52 of 1987
The Bahamas	NR	NR	NR	NR	Fisheries Regulations of 1986 Chapter 244
Barbados	NR	NR	NR	NR	No legal framework regulating QC
Belize	178	NR	NR	85 MC	Fisheries Act of Belize is Chapter 210 of the Laws of Belize Revised Edition 2000. Enacted in 1948 and last amended in 1989
Bonaire	PCS	PCS	PCS	PCS	Island Resolution Marine Park Bonaire (A.B. 2010, nr. 14) article 12
BVI	178	NR	Yes	226 D	Fisheries Regulations of 2003.
Cayman Islands	NR	NR	NR	NR	The National Conservation Law 2013, Part 2, Section 4.
Colombia	NR	NR	NR	100 MC	Law 13 of 1990 "General Fisheries Statute" and its Regulatory Decree 2256 of 1991, Law 915 of 2004
Costa Rica	PCS	PCS	PCS	PCS	Fisheries Act of Costa Rica 1948. Decree Article 1, No. 19203 MAG, 1989.
Cuba	200	8	Yes	NR	Decree-Law 164 (Fishing Regulations) 1996; Resolution 87 of 1996 (CITES Regulations); Decree- Law 200 of 2000 for environmental breaches; Resolution 220 of 2008; Resolution 160 of 2011 (Declared species of special significance in Cuba)
Curacao	180	NR	NR	225 D	Fisheries regulation (PB 1991, nr. 74)
Dominica	180	NR	Yes	225 D	2004 FISHERIES S.R.O (Regulations not gazetted thus not legally binding)

Dominican Republic	200	NR	NR	227 NI	Law 64-00, Decree 833-03 of 2003, Law 307 of 2004, decree 499-09 of 2009
Grenada	180	NR	Yes	225 D	Fisheries Regulations Statutory Rules and Order # 24 of 1996
Guadeloupe	NR	NR	NR	250 MC	decree n°2002-1249 of 19/08/2002
Jamaica	220	NR	Yes	NR	Fishing Industry Act of 1975; 1976 Regulation; e Fishing Industry (Conservation of Conch (Genus <i>Strombus</i>)) Regulations, 2000
Martinique	NR	NR	NR	250 MC	Decree n°99.4296 of 29/12/1999
Mexico	200	NR	NR	NR	Ley General de Equilibrio Ecológico y Protección al Ambiente (LGEEPA), la Ley General de Pesca y Acuicultura Sustentables (LGPAS), la Carta Nacional Pesquera y la NOM-013-PESC-1994,(DOF, 1995; DOF, 2010 y 2012).
Montserrat	NR	NR	NR	NR	No legal framework regulating QC
Nicaragua	200	9.5	Yes	NR	Decree DGRN No. 407--PA-05 of 2005
Panama	PCS	PCS	PCS	PCS	Resolución ADM/ARAP No. 017 de 22 de Mayo de 2015
Puerto Rico	229	9	Yes	NR	Reglamento de Pesca de Puerto Rico 2010 No 7949. Artículo 8.22-23
Saba	190	NR	Yes	NR	Marine Environment Ordinance Saba (A.B. 1987, no. 10) article 6
St Barthélemy	190	7	Yes	250 MC	Délibération du Conseil Territorial N°2015-035 du 27 juillet 2015, article 13 and 42
USVI	229	9.5	Yes	NR	V.I.C., Title 12, Chapter 9A, §316-1 & 316-4 and 50 CFR 622.3, 50 CFR 622.4
St Eustatius	190	NR	Yes	NR	Marine Environment Ordinance (A.B. 1996, No. 03) article 7
St Kitts and Nevis	180	NR	NR	225 D	Fisheries regulation (PB 1991, nr. 74)
St Lucia	180	NR	Yes	280 D	Statutory Rules and Orders Act Part 6 Sec 18 of 1986
St Maarten	178	NR	NR	NR	Fisheries regulation (PB 1991, nr. 74)
St Martin (French)	NR	NR	NR	250 MC	Decree n°2002-1249 of 19/08/2002

St Vincent & the Grenadines	180	NR	Yes	225 D	Fisheries Regulation 1987, SRO, Chapter 6, Sec 18
Trinidad & Tobago	NR	NR	NR	NR	No legal framework regulating QC
Turks & Caicos	180	NR	NR	226.8 D	Fisheries Protection Ordinance Chapter 10.08 2009
USA (Florida)	PCS	PCS	PCS	PCS	Art. IV, Sec. 9, Fla. Const. Law Implemented Art. IV, Sec. 9, Fla. Const. History—New 6-17-85, Amended 12-11-86, 1-1-91, 7-15-96, Formerly 46-16.001, Amended 9-1-13.

Table S2.3. Overview of closed season for queen conch. Dark grey = closed season, white = open season, and light grey = open season, closed for export only. Data from legislation presented in Table S2.2.

Location	Month												Closed season
	J	F	M	A	M	J	J	A	S	O	N	D	
Anguilla													No closed season
Antigua & Barbuda													1 July - 31 Aug
Aruba													Permanent closed season
The Bahamas													No closed season
Barbados													No closed season
Belize													1 July - 30 Sept
Bonaire													Permanent closed season
British virgin islands (BVI)													15 Aug - 31 Oct
Cayman Islands													1 May - 31 Oct
Colombia (San Andrés)													1 Jan - 19 May and 1 Jun - 31 Oct
Colombia													1 Jun - 31 Oct
Costa Rica													Permanent closed season
Cuba													1 May - 30 Sept
Curacao													No closed season
Dominica													No closed season
Dominican Republic													1 July - 31 Oct
Grenada													No closed season
Guadeloupe (0-25m)													1 Jan - 30 Sept
Guadeloupe (>25m)													1 Feb - 30 Sept
Martinique													No closed season
Jamaica													31 July - 1 Feb (>30 m closed)
Mexico (Campeche)													1 Jan - 14 Mar and 16 July - 31 Dec
Mexico (Chinchorro Bank)													Permanent closed season
Mexico (Quantino Roo)													1 May - 31 Oct
Mexico (Yucatan)													Permanent closed season
Montserrat													No Closed season
Nicaragua													1 Jun - 30 Sept
Panama													Permanent closed season
Puerto Rico													1 Aug - 31 Oct
Saba Island													No closed season
St Barthélemy													1 April - 31 Sept
USVI													1 Jun - Oct 31 (or TAC)
St Eustatius													No closed season
St Kitts & Nevis													No closed season
St Lucia													No closed season
St Maarten													No closed season
St Martin (French)													1 Feb - 31 Aug
St Vincent & the Grenadines													No closed season
Turks & Caicos													15 Jul - 15 Oct (exports only)
Trinidad & Tobago													No closed season
USA (Florida)													Permanent closed season

Table S2.4. Overview of spatial variation in reproductive season for queen conch (*Lobatus gigas*) in the Wider Caribbean Region based on observed reproductive behavior (copulation, egg-laying, presence of solitary egg-mass). Color indicate the relative activity of observed reproductive behaviors (black = high activity of reproductive behaviors, grey = medium activity of reproductive behaviors, light grey = low activity of reproductive behaviors (<1%), white = no reproductive behaviors observed, diagonal bars = no data available). Sites are arranged from north to south. * = true extent of reproductive season could not be fully assessed due to lack of data or low densities of flared-lip (adult) conch in the survey area.

Location	Months												Months	Lat	Long	Reference
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec				
Bermuda													4.5	32.3	-64.8	Berg et al., 1992
Florida													4.5	25.7	-80.1	D'Asaro, 1965
The Bahamas													4.5	23.5	-77.8	Stoner et al., 1992
Mexico													8	22.3	-89.7	Perez & Aranda, 2002
Turks & Caicos													6	21.9	-72.1	Davis et al., 1984
Turks & Caicos													6	21.8	-72.1	Davis et al., 1987
Cuba													12	18.8	-77.7	Cala et al., 2013
Jamaica													*6	18.5	-77.4	Salley, 1986
St John (USVI)													8	18.3	-64.7	Randall, 1964
Puerto Rico													*5	17.9	-67.0	Appeldoorn, 1993
St Croix													9	17.8	-64.8	Coulston et al., 1987
Saba													*4	17.6	-63.3	This study
St Eustatius													9	17.5	-63.0	This study
St Kitts													*3	17.3	-62.6	Wilkins et al., 1987
St Kitts													*5.5	17.3	-62.6	Buckland, 1989
Barbados													8	13.2	-59.4	Bissada, 2011
Venezuela													5.5	11.8	-66.8	Brownell, 1977
Venezuela													7.5	11.8	-66.7	Weil & Laughlin, 1984

Chapter 3

Underwater Towed Video: A Novel Method to Estimate Densities of Queen Conch (*Lobatus gigas*; Strombidae) Across Its Depth Range

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Abstract

Queen conch (*Lobatus gigas*) populations living deeper than 20 m are rarely studied, because of the limitations of conventional survey methods using divers [i.e., belt transect (BT), towed-diver]. A crucial management goal for conch populations is to maintain adult densities at adequate levels to ensure reproduction, which is highly density dependent. Therefore, accurate estimates of adult conch densities, both in shallow and deep areas, are essential. The rapid technical progress of video systems has made it possible to develop new cost-effective ecological sampling tools, which can be used to survey areas previously hardly accessible. A lightweight towed video array was used, which was able to survey adult conch throughout the species entire depth range (ca. 0–60 m depth), in a safe and efficient manner. The towed video method (TVM) was compared with a conventional BT method using scuba divers, in its ability to identify adult live and dead conch. A series of intercalibration transects were conducted in a high-complexity (HC) and a low-complexity (LC) habitat by having the towed video followed by a diver conducting a concurrent standard BT, covering the same surface area as the towed video. In both the HC and LC habitat, adult live queen conch had similar counts with both methods. Adult dead conch were not mistaken for live conch but were significantly underestimated with the towed video compared with the BT. The results validate the use of TVM as a reliable sampling tool to estimate densities of live adult conch in both HC and LC habitats throughout the species' depth range.

3.1 Introduction

Queen conch [*Lobatus gigas* (Linnaeus, 1758)] is a large marine gastropod, which is widely distributed throughout the Caribbean region, where it supports one of the most important fisheries (Appeldoorn 1994, Acosta 2002). It has been heavily exploited throughout large parts of its distribution range (Stoner 1997, Acosta 2002), which resulted in concerns for the species' future and its listing in Appendix II of the Convention on International Trade of Endangered Species of Wild Fauna and Flora in 1992. Queen conch occurs to a depth of 60 m (Randall 1964) but is most commonly found from 0 to 25 m (Ehrhardt & Valle-Esquivel 2008). Although most common in depths below 25 m, deep-water (> 25 m) conch populations can be found

throughout the Caribbean, for example, Bahamas, Belize, Martinique, Turks and Caicos, Jamaica, Puerto Rico (Berg Jr 1975, Berg & Olsen 1989, Stoner & Sandt 1991, Appeldoorn 1997, Reynal et al. 2009, García-Sais et al. 2012). Unfished deep-water adult populations are believed to provide significant recruitment to shallow-water stocks and are considered critical spawning stock refugia (Appeldoorn 1997). Such unfished deep-water populations are sometimes assumed to exist and to replenish fished shallow-water populations without proper confirmation of their presence (MRAG 2013). A crucial management objective for queen conch populations is to maintain adult densities at adequate levels to ensure successful reproduction (Appeldoorn et al. 2011), as reproductive success in queen conch is highly dependent on adult conch densities (Stoner & Ray-Culp 2000). Stoner and Ray-Culp (2000) demonstrated that mating and spawning behaviors reach maximum levels at approximately 200 conch per ha and that reproductive behavior decreases below this level until they cannot be observed below 48 conch per ha. Furthermore, the Queen Conch Expert Workshop Group Report (2012) advised setting harvest quota at no more than 8% of the adult conch stock. Accurate estimations of live adult conch densities, in shallow and deep areas alike, are thus essential to set sustainable harvest quotas. Because of safety limitations of survey methods using scuba, areas below 20 m are rarely surveyed and areas below 30 m are generally excluded from biomass estimates (Queen Conch Expert Workshop Group Report 2012, MRAG 2013). In addition, dive surveys can be logistically demanding and relatively expensive, in particular when remote offshore areas are surveyed (Queen Conch Expert Workshop Group Report 2012). The rapid technical progress of video systems has made it possible to develop new cost-effective sampling tools to study benthic organisms, beyond depths safe for diving, using video technology (Stevens 2003, Sheehan et al. 2010). This study describes the suitability of a light and affordable towed video method (TVM) to determine densities of adult (flared lip) queen conch throughout its entire depth range (ca. 60 m depth), in a safe and cost-effective manner. This study aims to assess the ability of the TVM to (1) correctly identify live adult queen conch, and (2) accurately estimate adult queen conch densities. Simultaneous towed video and diver belt transects (BT) were conducted to test the following hypothesis: the TVM does not differ in its ability to accurately estimate live adult queen conch densities from conventional methods using scuba in high-complexity (HC) and low-complexity (LC) habitats.

3.2 Materials and Methods

3.2.1 Study Site

St. Eustatius is a small island (21 km²) located between 17° 28' and 17° 32' N latitude and 62° 56' and 63° 0' W longitude (Figure 3.1). The St. Eustatius National Marine Park (SNMP) surrounds the entire island and extends from the high-tide level to a depth of ca. 30 m. The total surface area of the SNMP is 27 km². The Marine Park, which includes the Northern Reserve (163 ha) and the Southern Reserve (364 ha), was established in 1996. In these two reserves, fishing or anchoring is not allowed. Low-relief gorgonian reefs amount to 22% of the SNMP and are concentrated to the shallow (< 20 m) eastern part of the island. The reef habitats and seagrass beds are concentrated at depths of about 24 m and each cover ca. 4% of the SNMP. Rocky reef areas are limited to the southern and southwestern shelf areas, whereas seagrass beds are confined to the north (Debrot et al. 2014).

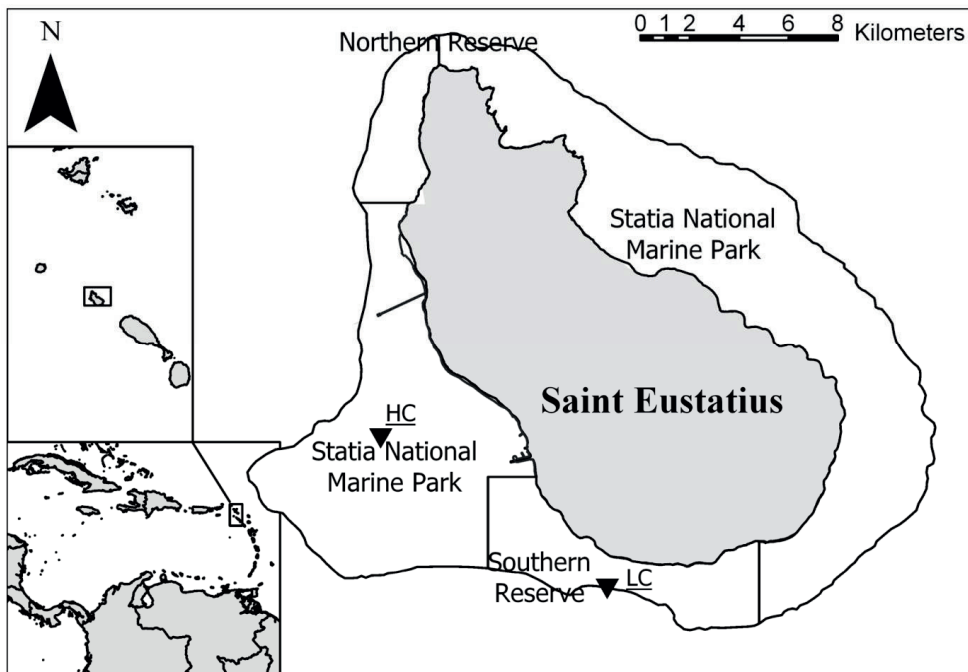


Figure 3.1. Location of St. Eustatius in the northeastern Caribbean.

3.2.2 Towed Video Method

The hovering towed video array was based on a design of Stevens (2003) and Sheehan et al. (2010) and consisted of a polyvinyl chloride (PVC) frame with a live view camera (Seaview super mini; Seaviewer Cameras Inc., Tampa, FL, www.seaviewer.com) and a transect camera (GoPro Hero 2; GoPro, San Mateo, CA, www.gopro.com) (Figures 3.2A and 3.3). The live view camera was mounted on the frame in a forward and downward (30°) position, sending a real-time feed to the operator on the boat through a cable. The feed was continuously monitored by the operator to avoid high-relief habitat and to adjust for changes in depth. The transect camera was mounted on the frame in a forward and more downward (45°) position than the live view camera. This position allowed for an optimal angle for video analysis. The 1 m width of the transect was indicated by two green lasers (Z-bolt SCUBA-1 Underwater green laser; Beam of Light Technologies Inc., Happy Valley, OR, www.z-bolt.com) mounted parallel on a PVC bar on top of the frame at a fixed distance of 1 m apart. The two green lasers were angled in the same forward and downward position as the transect camera. The laser dots were visible on the video recordings (Figure 3.2B).

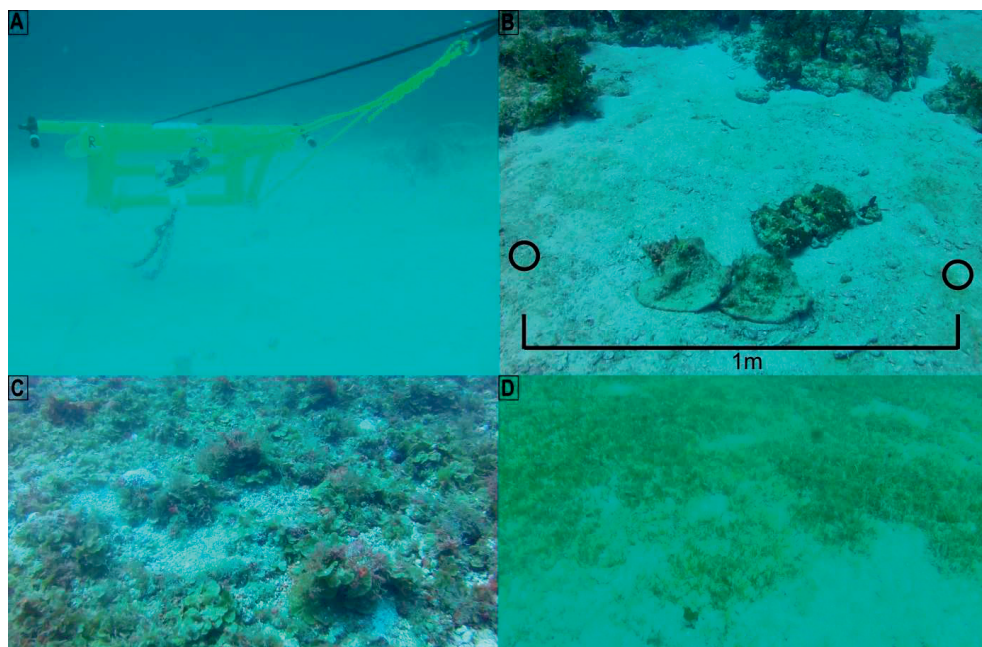


Figure 3.2. (A) Towed video array surveying in sand habitat; (B) screenshot from transect camera recordings, two adult paired queen conch, laser dots inside black circles indicate the outer points of

the 1m width of the transect; (C) screenshot from transect camera recordings of the HC habitat consisting of rubble and algae; and (D) screenshot from transect camera recordings of the LC habitat consisting of sand, seagrass, and algae.

Two sealed and air-filled PVC pipes attached on top of the frame kept the array slightly positively buoyant. Total weight of the array including cameras and lasers was < 10 kg. A length of chain attached to the bottom of the frame secured the downward position of the frame and allowed the array to hover over the bottom. The array was towed behind a 6 m long vessel with a 115 hp outboard engine, using a tow-line, which was connected to the frame. The length of the towline was adjusted depending on depth. A 10 kg drop weight was attached to the towline 10 m in front of the frame to ensure that the frame was kept in a horizontal forward position and helped to absorb some of the movements caused by surface waves. The drop weight was kept at a height of 1–2 m above the seafloor and by adjusting the height of the drop weight above the seafloor, the array was kept at the preferred height of ca. 1 m above the substrate.

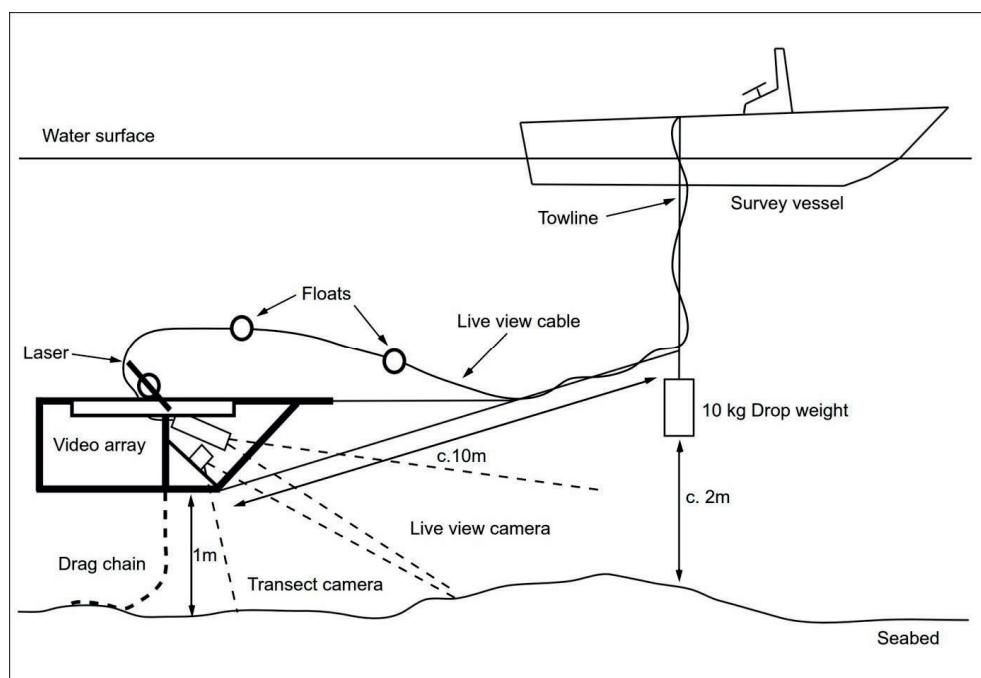


Figure 3.3. Arrangement for deployment of the towed video array (not to scale).

3.2.3 Calibration Transects

The calibration of the TVM was conducted in two different benthic habitats with different levels of complexity and where adult queen conch commonly occurred. The HC habitat, found at a depth range of 20–33 m, consisted of rubble and algae (Figure 3.2C) and the LC habitat, found at 10–26 m depth, consisted of sand, seagrass, and algae (Figure 3.2D). Habitat complexity was expected to potentially influence results of the TVM, because, based on previous experience, adult queen conchs are slightly more difficult to identify in HC habitats. During the calibration transects, an experienced conch research diver used to identify adult conch in different habitats, followed the towed video array and at the same time conducted a standard BT (CRFM 2013) covering exactly the same area (equal width and length) as the transect made by the TVM. Belt transects are a common method used to estimate the distribution of organisms within a specified area. All individuals of the species of interest are recorded within an area with a set length and width (CRFM 2013). The towed method was operated by two individuals, the first monitored the live view feed and the second adjusted the length of the towline to keep the array at the preferred height above the bottom. The video array was towed at low speeds (0.25–0.5 m/s) or when possible, the vessel drifted with the current and/or the wind. Keeping the speed lower than 0.5 m/s was critical as at higher speeds, the transect video recordings became blurry and unsuitable for analysis. The 1 m width of the transect was indicated by the lasers attached to the towed video array. To have the same transect length for both methods, hand signals in front of the video camera and live view camera by the diver were used to indicate begin and endpoint in the video recordings. Based on the divers hand signals, visible through the live view feed, global positioning system points of the begin and endpoint were determined with a handheld global positioning system (Garmin GPSmap 78, Garmin Ltd., Olathe, KS, www.garmin.com), which was set to track the position every 10 sec to accurately follow the transect and calculate transect length. Begin and end depths were also recorded for each transect and determined by the vessel's depth sounder. The video recordings of the transects were saved on secure digital cards and converted to audio video interleave-files using Xilisoft video converter (Xilisoft Corporation, San Diego, CA, www.xilisoft.com). All adult conch inside the transect as well as all adult conch which were only partially inside the transect were counted by the diver conducting the BT and the video analyst. After conversion, videos were analyzed for the occurrence of adult queen

conch by an independent researcher not involved in the BT to avoid bias, using TransectMeasure computer software (SeaGIS Pty. Ltd., Bacchus Marsh, VIC, Australia, www.seagis.com.au). To distinguish live from dead conch, the diver turned over each animal in the transect, whereas the video analyst determined live from dead based on visual cues (e.g., the position of the conch, damage to shell, tracks on the sea bottom, and movement).

3.2.4 Statistical Analysis

To test the difference between the two survey methods, the conch counts (X , in numbers) were first transformed to $X' = \sqrt{X + 0.5}$, which is the preferred method in case of dealing with small numbers, including zero values in the data (Zar 1996). The transformed counts were first tested for differences using a Student's paired t-test, if their variances were not significantly different ($P > 0.05$) according to a Levene's test (which was never the case). Next, a major axis regression was carried out to investigate the relationship between the results of both survey methods. This method is preferred if neither of the variables can be considered independent, when they are in the same units and if the variance of error is approximately the same for both variables (Legendre 2013). Data analysis was performed using the software environment R (R Development Core Team 2016) and the packages *car* and *lmodel2*.

3.3 Results

Eighteen transects were conducted in the HC habitat and 19 transects in the LC habitat covering a total area of 1.8 ha. Transects varied in length from 130 to 982 m and ranged from 10 to 33 m in depth. In the HC habitat, both survey methods recorded similar counts of adult live queen conch (Table 3.1), with mean counts of 31.94 with the BT and 30.06 with the TVM (paired t-test: $t = 1.623$, $df = 17$, $P = 0.122$). In the LC habitat, adult live queen conch counts were also similar with both methods (Table 3.1), with mean counts of 6.94 with the BT and 6.84 with the TVM (paired t-test: $t = 0.90$, $df = 18$, $P = 0.380$). In addition, the major axis regression showed that the numbers of adult live conch recorded by the TVM and BT in the HC habitat were similar (slope not significantly different from one) and had a high explanatory level ($R^2 = 0.89$) (Table 3.2, Figure 3.4A). In the LC habitat, both methods were even more similar (slope not significantly different from one) with a very high explanatory level ($R^2 = 0.99$)

(Table 3.2, Figure 3.4B). Adult dead conch counts were consistently underestimated with the TVM (Table 3.1) in the HC habitat, with mean counts of 5.72 with the BT and 2.22 with the TVM (paired t-test: $t = 4.97$, $df = 17$, $P < 0.05$). No dead adult conchs were found in the LC habitat (Table 3.1). The number of recorded adult dead conch in the HC habitat was lower with the TVM than with BT (slope significantly different from 1) with only a moderate explanatory level ($R^2 = 0.50$) (Table 3.2, Figure 3.4C).

Table 3.1. Summary of the result from the calibration transects in the HC and LC habitat with the TVM counts and the divers' counts during the BT [counts, mean counts per transect, SD, and the number of

Group	Habitat	Method	# T	Count	Mean Count	SD	0-T
Adult live	HC	BT	18	575	31.94	12.62	0
	HC	TVM	18	541	30.06	10.84	0
Adult live	LC	BT	19	132	6.94	4.97	2
	LC	TVM	19	130	6.84	4.94	2
Adult dead	HC	BT	18	103	5.72	4.79	1
	HC	TVM	18	40	2.22	2.01	4
Adult dead	LC	BT	19	0	0	0	19
	LC	TVM	19	0	0	0	19

transects with 0-counts (0-T)].

Table 3.2. Summary of the results from the major axis regression analysis of the squared root ($X + 0.5$) transformed TVM counts and the squared root ($X + 0.5$)-transformed BT counts in the HC and the LC habitat [lower (lower CI) and upper confidence interval (upper CI) of the slope] (* = significant).

Group	Habitat	Mean slope	Lower CI	Upper CI	R ²	p
Adult live	HC	0.87	0.72	1.05	0.89	$p < 0.01^*$
	LC	0.99	0.95	1.04	0.99	$p < 0.01^*$
Adult dead	HC	0.57	0.30	0.93	0.50	$p < 0.01^*$

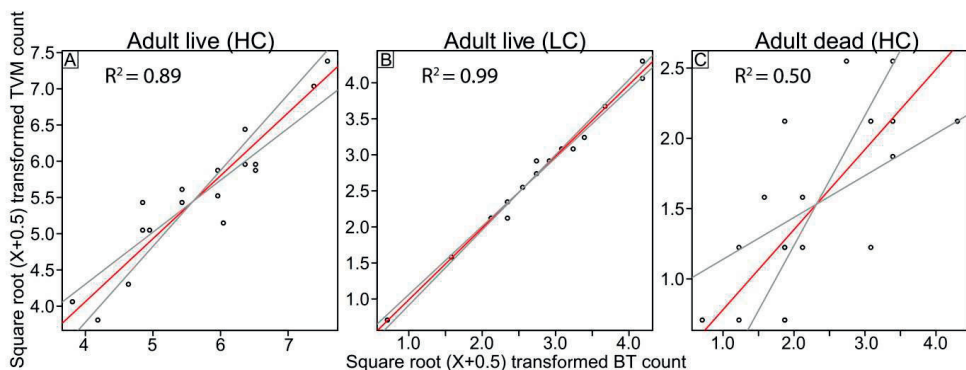


Figure 3.4. Major axis regression of the square root ($X + 0.5$)-transformed TVM (X variable) counts and the BT counts (Y variable) of adult (flared lip) live conch in the HC and LC habitat and dead adult conch in the HC habitat and the presence of dead conch do not.

3.4 Discussion

In this study, a lightweight and effective towed video array was developed capable of surveying adult queen conch populations in both HC and LC habitats throughout the species' entire depth range (ca. 0–60 m). The TVM proved to be effective in accurately identifying live adult conch, in both the HC and the LC habitats tested, and both methods yielded overall the same counts. Especially in the LC habitat, the results of both methods appeared interchangeable (Figure 3.4A). This also applied for the HC habitat, although the variability in the estimates was larger, resulting in more spread around the intercalibration line and a slightly lower R^2 (Figure 3.4B). It is likely that the slightly larger variability in the more complex habitat is caused by the overall greater difficulty in detecting conch in this habitat. Accurately distinguishing between live and dead conch is important to produce reliable density estimates, which is critical for queen conch management (Ehrhardt & Valle-Esquivel 2008). Concerns have been raised that the identification of live versus dead conch could be a potential problem for the TVM described here (CRFM 2013). The results from the major axis regression (Figure 3.4A, C) of the adult live and dead conch in the HC habitat showed that identification of live versus dead conch is accurate for the TVM. Live conchs are not mistaken for dead conch and the presence of dead conch do not lead to overestimates of live conch. Thus, the results prove that the concerns raised by CRFM (2013) regarding the accuracy in the identification of live

versus dead conch are unfounded. Towed video method counts of dead adult conch in the HC habitat were consistently underestimated compared with the BT counts (Figure 3.4C). Shell erosion in conch start already when the conch is alive and is affected by the type of stratum the conch occupies (Medley 2008). The rate of erosion is highest in hard and rocky bottoms and continues after the conch is dead (Medley 2008). The slowly degraded shells of dead adult conch made them more difficult to differentiate from the complex structures (i.e., rubble) of the HC habitat. The difficulty of identifying these partly degraded shells was likely the cause of the consistent underestimation of dead adult conch by the TVM, which was not able to identify these conch in contrast to the diver conducting the concurrent BT. Although underestimations of dead adult conch with the TVM is evident, this does not pose a major obstacle for the TVM as the primary focus for management is the ability to estimate densities of live adult conch accurately. The key advantage of the TVM over other conventional survey methods using scuba is the ability to accurately determine live adult conch densities throughout the species entire depth range (ca. 0 – 60 m), with essentially unlimited bottom time (Stevens 2003, Spencer et al. 2005). The two most common methods to study the abundance and distribution of queen conch populations are towed scuba diver (Wood & Olsen 1983, Stoner et al. 2012) and BT by scuba divers (Clark et al. 2005, CRFM 2013). Both methods use scuba and are thus restricted to limits of safe diving, although for practical reasons areas below 20 m are rarely surveyed with methods using scuba (Queen Conch Expert Workshop Group Report 2012). During this study, the TVM was only used to a depth of 33 m to be able to conduct the concurrent BT. However, the TVM has been used to determine adult queen conch densities to a depth of 57 m (unpublished data) on the Saba Bank, an offshore bank located ca. 20 km west of St. Eustatius. With methods using scuba, costs increase with survey depth (Prada et al. 2014), however, the TVM is equally cost-effective at any depth. Further advantages of the TVM is its capability of covering large distances (Sheehan et al. 2010) and make permanent records of the surveys (Spencer et al. 2005), which can be reanalyzed if necessary. Moreover, the recorded transects can be used to quantify habitat characteristics on a fine and broadscale (Spencer et al. 2005, Sheehan et al. 2010). The TVM was accurate in identifying adult live conch in both the HC and LC habitat with a high R^2 in both habitats (Figure 3.4A, B). In high-relief habitats, such as coral reefs, the TVM is, however, not capable of safely navigating. Although these areas are not usually preferred by queen conch (Acosta 2006),

conch surveys in such areas will require methods using divers. Controlling the speed over the seafloor is also important, with the transect camera (GoPro Hero 2) used in this study, speeds above 0.5 m/s resulted in blurry video unsuitable for quantitative analysis. The results of this study verify the TVM ability in making accurate density estimates of live adult queen conch. With this verification and the advantages of the TVM, especially at greater depth, over conventional survey methods using divers, managers now have a new survey tool at their disposal. The entire depth range of adult queen (0 – 60 m) can be surveyed and accurate density estimates of live adult conch can be made with the TVM, without an increase in costs or risks to field staff.

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Chapter 4

Spatial dependency in abundance of Queen conch, *Lobatus gigas* in the Caribbean reveals the importance of surveying deep-water distributions.

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Abstract

Queen conch (*Lobatus gigas*) is an economically and culturally important marine gastropod, which has been subject to over-exploitation throughout most of its geographical range. However, knowledge about drivers of abundance and distribution of the species remain incomplete, especially in the deeper parts of its range (25 – 60m). By combining a recently developed towed-video system able to estimate adult queen conch densities across the species' entire depth range with a conventional belt-transect method, a more comprehensive survey of conch abundance was performed at three sites in the Eastern Caribbean (Anguilla, St Eustatius, Saba Bank). Bayesian hierarchical spatial models (Integrated Nested Laplace approximations) modeled distribution patterns of adult conch revealing patchy distributions, caused by spatial dependency. This dependency is most likely related to aggregating behavior during mating events. Environmental variables, such as algae cover, distance to the open ocean, and depth showed important non-linear effects on conch abundance, although these differed among sites. The proportion of reef and sand cover had important negative effects on conch abundance at all sites. High densities (>100 /ha) of adult conch were found only at depths >17 m at all three sites. The lack of strong generic location over-crossing relationships between abiotic and biotic factors and adult conch abundance and distribution is likely partly due to this spatial dependency, and location-specific factors that affect different stages of the conch's life-history. Intermediate and deep areas (ca. 17 – 45 m) contain most of the reproductive output of conch in the survey areas and are highly important for reproductive capacity. Thus, surveying areas at depths beyond the practical limitation of divers is of great importance to obtain more reliable population estimates.

4.1 Introduction

Queen conch (*Lobatus gigas*), is an economically and culturally important marine gastropod (Appeldoorn 1994, Brownell & Stevely 1981) found throughout the Wider Caribbean Region and the southern Gulf of Mexico. Conch have biological characteristics (e.g. slow-moving, often occurring at shallow depths, and aggregating during the reproductive season), which make it vulnerable to overfishing (Appeldoorn et al. 2011). The species has been over-

exploited throughout large parts of its range (Acosta 2002, Stoner 1997), resulting in concerns for the species' future and its listing in Appendix II of the Convention on International Trade of Endangered Species of Wild Fauna and Flora in 1992.

Although conch are most common in depths <25 m (Weil & Laughlin 1984, Ehrhardt & Valle-Esquivel 2008), the species can be found up to depths of 60 m (Randall 1964). The depth range of conch is believed to be restricted primarily based on light attenuation, which limits their photosynthetic food sources (e.g. filamentous alga) (Randall 1964, Ray & Stoner 1994, Creswell 1994). Conch move to deeper waters with age and size (Randall, 1964, Weil & Laughlin 1984), and consequently adult conch have, on average, a deeper distribution than juveniles. Unfished deep-water adult populations are believed to provide significant recruitment to shallow-water stocks and are considered critical spawning stock refugia (Appeldoorn 1997). Deep-water populations (>25 m) have been found in the Caribbean (e.g. the Bahamas, Belize, Martinique, Turks and Caicos, Jamaica, Puerto Rico [Berg Jr 1975, Berg & Olsen 1989, Stoner & Sandt 1991, Appeldoorn 1997, Reynal et al. 2009, García-Sais et al. 2012]), but these populations have rarely been studied in detail. Deep population are sometimes assumed to exist and replenish fished shallow-water populations, yet without proper confirmation of their presence (MRAG 2013). The depth range of conch can also be influenced by fishing pressure. When adult densities in shallow depths are reduced, the remaining conch are often found at greater depths where they are more difficult to reach by fishermen (Stoner & Schwarte 1994, Stoner et al. 2018).

Adult conch can be found in a range of habitats (Stoner et al. 1994), with a preference for sand/algal flats, but they are also found on hard bottom habitats such as coral rubble (Torres-Rosado 1987, Acosta 2001, Stoner & Davis 2010). They are rarely found on soft bottoms (silt/mud) or in areas with high coral cover (Acosta 2006), likely due to restrictions in their movement in such habitats (Dujon et al. in press). Tidal channels with high water flow have also been shown to be of ecological importance to conch, with increasing abundance of conch of all sizes found closer to the tidal channel (Kough et al. 2017). Due to the positive association of areas with high water flow (i.e. tidal channels) with conch, it could be expected that other areas with high water flow, such as around the edges of offshore banks, would have a similar effect on conch abundance (Hamner & Hauri 1981).

The above relationships between environmental variables and conch distribution and abundance were mostly established through dive surveys. Due to the safety limitations of surveying using scuba, areas deeper than 20 m are rarely surveyed, and areas below 30 m are generally excluded from biomass estimates (Queen Conch Expert Workshop Group Report 2012, MRAG 2013). In addition, dive surveys are logistically demanding and relatively expensive, particularly when surveying remote offshore areas (Queen Conch Expert Workshop Group Report 2012). The rapid technical progress of video systems has made it possible to develop new cost-effective sampling tools to study benthic organisms beyond depths safe for diving (Stevens 2003, Sheehan et al. 2010). A towed video method capable of accurately determining densities of live adult conch throughout the species' depth range (0 - 60 m) was developed by Boman et al. (2016), which facilitates assessing the abundance and distribution of adult conch at deep and offshore locations. This also enables the study of the relationships between environmental variables and conch abundance throughout its depth range which is, until now, poorly studied.

A complicating factor when studying the relationships between environmental variables and conch distribution and abundance is the tendency of conch to aggregate, especially during the reproductive season (Glazer & Kidney 2004). A patchy distribution pattern with spatial auto-correlation among locations is thus to be expected (Kough et al. 2017, Vallés & Oxenford 2012). Therefore, it is important to use statistical tools which account for auto-correlative spatial patterns, as well as responses to various biotic and abiotic variables (Keitt et al. 2002, Carroll & Pearson 2000, Zuur et al. 2017). In this study, Bayesian hierarchical spatial models using Integrated Nested Laplace approximation (INLA) were used to account for both aspects.

To predict adult conch distribution and abundance throughout the species' entire depth range, surveys were conducted at three sites in the Eastern Caribbean with varying environmental characteristics, thus providing an opportunity to examine how biotic and abiotic factors influence the abundance and distribution patterns of adult conch. This study aims to (1) evaluate how the spatial distribution of adult queen conch varies in response to a number of known biotic and abiotic variables within and between sites which vary in environmental conditions; (2) evaluate whether patchy distribution and spatial dependency observed in previous studies is a general pattern in queen conch, and what causes it; and (3)

test the hypothesis that significant densities of adult conch are a common occurrence in deep areas (>25 m), requiring a depth extension of conch surveys that are used to provide advice on fishing quotas.

4.2 Methods

4.2.1 Study area and survey design

Surveys were conducted at three different sites in the Eastern Caribbean (Anguilla, St Eustatius [islands with a narrow continental shelf], and Saba Bank [offshore bank]) (Figure 4.1), which differed in environmental variables (e.g. habitat homogeneity, geomorphology, waterflow patterns, depth).

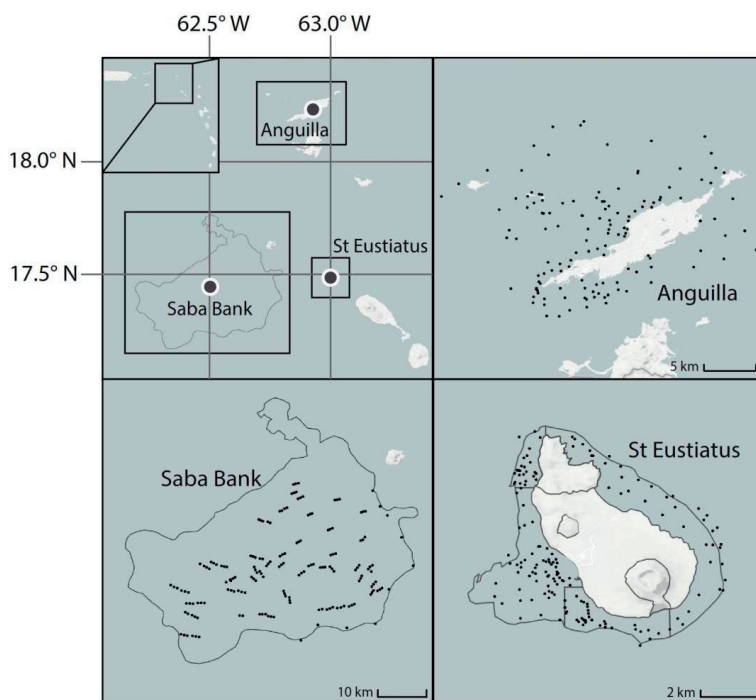


Figure 4.1. Map of survey areas (black dots represents transects conducted at the sites).

Anguilla (91 km²) (18.21°N, 17.04°W) is a low-lying coralline island situated roughly 260 km east of Puerto Rico and 7 km north of St Maarten. The island is surrounded by a mixture of patchy, barrier and fringing reefs interspersed with seagrass beds, sand channels and algal

flats (Wynne 2010). Survey transects (N = 132) were conducted between 31 August 2011 and 11 December 2015 in the waters around Anguilla, covering an area of approximately 940 km² in depths of 3 to 54 m, with 48% of transects (N = 63) conducted during the peak breeding season (May-September) (Table 4.1) (Boman et al. 2018). The area around Anguilla was divided into 5 × 5 km grid cells. Within each grid cell, 5-6 survey transects were dispersed randomly (N = 117). In addition, within the survey area, a total of 15 fixed transects were added for long-term monitoring of conch abundance in near shore shallow areas around Anguilla.

St Eustatius is a small volcanic island (21 km²) (17.49°N, 62.47°W) located 12 km south west of St Kitts & Nevis and 45 km north of St Maarten. The St Eustatius National Marine Park (SNMP) surrounds the entire island and extends from the high-tide level to a depth of 30 m. The total surface area of the SNMP is 27 km². Low-relief gorgonian reefs amount to 22% of the SNMP and are concentrated in the shallow (<20 m) eastern part of the island. The reef habitats and seagrass beds (mainly *Halophila stipulacea*) are concentrated at depths of about 24 m and each cover ca. 4% of the SNMP (Debrot et al. 2014). Rocky reef areas are limited to the southern and southwestern shelf areas, whereas seagrass beds are confined to the west and north (Debrot et al. 2014). Survey transects (N = 167) were conducted in and in close vicinity of, the SNMP at depths of 6.5 to 45 m between 11 June 2013 and 5 March 2014, with ca 62% of transects (N = 103) conducted during the peak breeding season (May-September) (Table 4.1) (Boman et al. 2018). Sampling locations in the waters surrounding St Eustatius were selected through a random sampling design (Ehrhardt & Valle-Esquivel 2008).

Saba Bank (17.4°N, 63.5°W) is a 2200 km² submerged bank located 4 km east of the island of Saba (17.63°N, 63.24°W) (Figure 4.1). Along the eastern edge an actively growing coral reef zone is present (Van der Land 1977, van Beek & Meesters 2014). The central, lagoon-like, part of the bank alternates between several habitat types: bare sand, patch reefs, rubble reefs, and sand or pavement (hard substrate) with macro-algae, sponges, gorgonians and/or coral structures (Lundvall 2008, Meesters 2010, Toller et al. 2010). Survey transects were made on the Saba Bank at depths of 16-57 m between 9 April 2013 and 12 November 2014, with ca 42% of transects (N = 72) conducted during the peak breeding season (May-September) (Table 4.1) (Boman et al. 2018). Sampling locations on Saba Bank were assigned randomly inside a

systematic grid (5 × 5 km cells). In addition, 10 fixed locations monitoring the long-term status of the coral reef along the eastern edge of the bank were added. Due to the size of Saba Bank (2200 km²), for practical reasons three transects were towed in short distance of each other, separated by roughly 500 m.

Table 4.1. Survey transect summary for the three sites.

Site	No. transects	Mean transect size (m ²)	Size survey area (km ²)	Mean survey depth (m)
Anguilla	132	635 (SD = 359)	940	22 (SD = 11.2)
St Eustatius	167	1450 (SD = 1800)	27	21.3 (SD = 7.1)
Saba Bank	172	525 (SD = 40)	2200	27.4 (SD = 7.2)

4.2.2 Abundance estimates

Adult conch (defined here as conch with a fully developed lip: [Boman et al. 2018]) abundance was estimated using two comparable methods i.e. standard belt transects (CRFM 2013) and a towed video method (Boman et al. 2016). Belt transects, using scuba, were primarily conducted in high-relief habitats (mainly reef habitats) in which the towed video array was less suitable to use (Boman et al. 2016). Total adult conch counts for each transect were calculated for the purpose of modelling conch abundance and distribution patterns. Density (number of conch/ha) estimates for each transect were also calculated but only used to compare with previous surveys and not used for the models.

Belt transects at each survey site were between 200-13900 m², with the majority of transects between 200 – 1500 m² (94 %), where all adult conch within each transect were counted. Difference in transect area surveyed within sites was accounted for in the models by adding it as a log transformed parametric covariate (sampling effort) (Table 4.2). Life status was determined by visual inspection after turning over the animal. The divers estimated the percentage cover of substrate (sand, rubble, reef) and macrobenthos (algae, seagrass) for each transect. The location of the transect was determined with a handheld GPS (Garmin GPSmap 78, Garmin Ltd., Olathe, KS, www.garmin.com). Mean depth was recorded for each transect, as determined by the diver's computer.

Towed video transects at all three sites were conducted following Boman et al. (2016), with a transect width of 1 m and a transect length of between 330 and 806 m. All adult conch inside the transects (i.e. more than 50% of shell inside the transect) were counted, and life status was determined based on visual cues (Boman et al. 2016). Substrate (sand, rubble, reef) and macrobenthos (algae, seagrass) cover were determined for each transect by analyzing 20 frames, with an equal spread in time over the transect. In each of the 20 frames, 10 set dots in a 2×5 pattern were overlaid, and the underlying substrate and macrobenthos was identified per dot. If a dot was blocked or the frame was blurry, the next frame in which substrate and macrobenthos for each dot could be determined was used. Substrate and macrobenthos cover for each transect was calculated based on the average total of dot points from the 20 frames. A handheld GPS system was set to track the position, every 10 sec, to follow the transect and calculate transect length accurately. Mean depth (m) was also recorded for each transect and determined by the vessel's depth sounder.

4.2.5 Bayesian hierarchical spatial models

4.2.5.1 Data exploration and model selection

The three sites had different geomorphology, with Anguilla and St Eustatius being islands with a narrow shelf and Saba Bank being an offshore submerged bank. Due to these differences in geomorphology, as well as tidal flow patterns, macro-habitat cover, and size of survey sites, relationships between conch counts and covariates were expected to vary among sites. Thus, each site was analyzed separately. Data exploration was applied, following the protocol described in Zuur et al. (2010). The observed conch data contained high percentages of zeros (60, 50, and 33 % for Anguilla, St Eustatius, and Saba Bank respectively), but this does not necessarily mean that zero-inflated models need to be applied. If a covariate in the selected model can explain the zeros in the data, applying a zero-inflated model is not necessary. To determine if a model can explain the zeros in the data, a simulation of data sets from the model (by sampling regression parameters from their posterior distribution) can be made, whereby the number of zeros for each simulated data set is counted and compared with the observed number of zeros in the original data (Zuur et al. 2016). If these are comparable there is no need to extend the Poisson Generalized linear models (GLM) or Generalized additive models (GAM) to zero-inflated models (Zuur et al. 2016).

Initial data exploration was carried out in order to determine (a) whether any of the covariates contained extremely large or small values, using Cleveland dot plots (Figure S4.1), and (b) whether there was collinearity among covariates, using variance inflation factors (VIF) and pairwise scatterplots (Table S4.1, Figure S4.2, S4.3, S4.4). Poisson GLMs were then fitted for all three sites for the analysis of count data, which were investigated for over-dispersion. All Poisson GLMs revealed over-dispersion, and thus identifying the cause of this is important (Hilbe 2011) as failure to do so can cause a bias in estimated parameters and too small standard errors (Zuur et al. 2017). A missing covariate can cause overdispersion, non-linear patterns, a large number of zeros in the data, and/or dependency between sampling locations (Zuur & Ieno 2016). Thus, model validation is required to identify the cause of over-dispersion.

Table 4.2 Summary and definition of covariates. The covariate Reef was used as a continuous variable (proportion of total substrate cover) for St Eustatius and as a categorical variable (absence – presence) for Anguilla.

Covariate	Definition
Rubble cover (P)	Proportion (P) of total substrate cover
Sand cover (P)	Proportion (P) of total substrate cover
Reef cover(P)	Proportion (P) of total substrate cover and Categorical (absence – presence)
Algae cover (P)	Proportion (P) of total macrophyte cover over the substrate
Seagrass cover (P)	Proportion (P) of total macrophyte cover over the substrate
Depth (m)	Mean depth (m) of transect
Distance to ocean (m)	Distance to open ocean or edge of Bank (> 100 m depth)
Sampling effort (m ²)	Log transformed total area (m ²) sampled during each individual transect
Latitude (dd)	Geographical coordinate of transect
Longitude (dd)	Geographical coordinate of transect

Scatterplots of conch counts versus each covariate were created to determine the presence of non-linear patterns. Covariates showing linear patterns were modelled as fixed effects, while covariates showing non-linear patterns were modelled as smoother functions (Poisson GAMs) to capture the non-linear patterns (Figure S4.5). If GAMs were still over-dispersed,

Pearson residuals were used to determine the presence of spatial correlation and, if these were present, GAMs with and without spatial correlation were applied (Zuur & Ieno 2018).

All models were estimated using Integrated Nested Laplace Approximation (INLA; Rue et al. 2009, Lindgren et al. 2011, Lindgren & Rue 2015, Zuur et al. 2017, Zuur & Ieno 2018). In INLA, the covariance matrix Ω of the spatially correlated random effects is quantified using the Matérn correlation function which makes dependency a function of distance and a set of unknown parameters. To obtain these parameters, a series of steps is carried out. First, a mesh is defined using a collection of small triangles. Greater numbers of triangles creates a finer mesh, and a more accurate the INLA solution. At the node of each triangle, a value w_k is estimated, using continuous domain stochastic partial differential equations (SPDE). These w_k s are directly linked to the spatially correlated random effect and together form the spatial random field, which is visualized by standard interpolation techniques (Zuur et al. 2017).

Spatial correlation represents either real dependency originating from population interactions or spatially structured environmental controls, or missing covariates. Models with and without spatial dependency were compared using the Watanabe-Akaike information criterion (Watanabe 2010), and the model with the lowest (best) WAIC value was selected. Besides the posterior mean of the spatial random field (w_k values), the posterior standard deviation for each w was estimated (based on a normal distribution), which can be used to infer which parts of the spatial random field are important (Zuur & Ieno 2018), with small standard deviation values ($0 \pm [2 \cdot \text{sd}]$) indicating a high importance.

One complicating factor for the Anguilla and St Eustatius data was the location of sampling locations were around an island, which meant that by default the spatial correlation crosses land. This is acceptable if the spatial correlation represents a missing covariate that affects sites on both sides of the island, but if it represents real dependency the model needs to take into account that conch do not cross land. This can be done with the barrier model (Bakka et al. 2018) that ensures that the spatial correlation matrix seeps around land and not across it. The choice between a model with and without the barrier should be based on biological relevance, and not only on statistical grounds, because barrier models tend to have larger WAIC values than standard models. Simulations of 10,000 data sets were conducted from the

model with the best fit to determine if these met assumptions and could cope with the number of zeros in the data (Zuur et al 2016).

Other technical details (i.e. specification of priors for hyperparameters and spatial correlation and mesh size) are provided in the supplementary material. All calculations were performed in R (R Core Team 2017) using the following packages: lattice (Sarkar 2008), sp (Pebesma & Bivand 2005), raster (Hijmans 2012), dismo (Hijmans et al. 2016), splancs (Rowlingson & Diggle, 2017), INLA (Rue et al. 2009), reshape (Wickham 2007), gstat (Pebesma 2004, Gräler et al. 2016), ggplot2 (Wickham 2016), ggmap (Kahle & Wickham 2013), maps (Becker et al. 2018), maptools (Bivand & Lewin-Koh 2017), mapdata (Becker & Wilks 2018), rgdal (Bivand et al. 2018), fields (Nychka et al. 2017), rgeos (Bivand & Rundel 2018), mosaic (Pruim et al. 2017), and mgcv (Woods 2017).

4.3 Results

4.3.1 General descriptive results

The observed conch data contained high numbers of transects with zero-counts: 132 (60%), 167 (55%), and 172 (33%) for Anguilla, St Eustatius, and Saba Bank respectively. Conch abundances reached up to 16 conch per transect for Anguilla, 42 for St Eustatius, and 45 for Saba Bank.

Saba Bank had the highest overall mean conch density (126/ha) of the three sites. Conch were found throughout Saba Bank from 16 m to 57 m depth, except along the eastern edge with high reef cover where no conch were found. Anguilla had the lowest overall mean density (27/ha) and conch were found around the entire island at depths of between 4 and 50 m in a range of habitats, with higher densities on the western and eastern coasts. St Eustatius had a mean density of 62/ha and conch were concentrated in the south-western and south-eastern parts of the island (Table 4.3). They were found in all habitats sampled, ranging from 11 to 45 m depth. The highest densities of conch were found in deep waters (> 25 m) at all three sites, with individual transects measuring densities of 393 conch/ha at a depth of > 40 m on Saba Bank, and densities of 311 and 285 conch/ha at depths just above 30 m at Anguilla and St Eustatius respectively.

Table 4.3. Summary statistics of adult conch densities (mean with 95 % confidence interval [CI]), at the three survey sites.

Overall mean density					
Location	Conch/ha	Lower CI	Upper CI	Max	No. Transects
Anguilla	26.5	16.6	36.3	335.6	132
Saba Bank	125.7	92.5	142.9	882.4	172
St Eustatius	61.6	43.1	80	950	167

Percentage of transects with conch					
Location	>0 conch/ha	>50 conch/ha	>100 conch/ha	>200 conch/ha	>300 conch/ha
Anguilla	39.90%	12.30%	9.40%	3.60%	2.20%
Saba Bank	66.80%	48.30%	35.50%	22.10%	12.20%
St Eustatius	51.50%	35.90%	23.40%	13.20%	7.20%

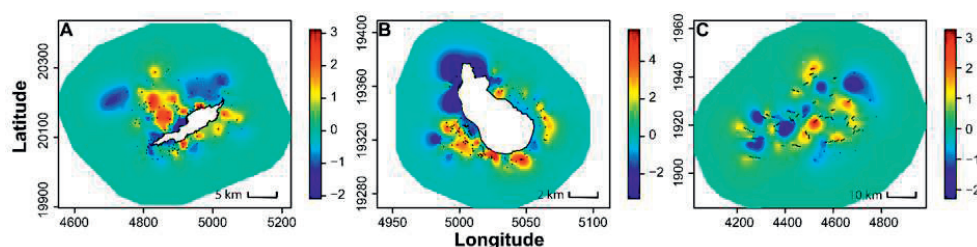


Figure 4.2. Spatial random field from the three survey sites (A = Anguilla, B = St Eustatius, C = Saba Bank) with areas of above average (red) and areas of below average (blue) conch abundance (counts of adult conch). Survey transect locations are indicated with black dots.

4.3.2 Bayesian hierarchical spatial models using INLA

4.3.2.1 Model selection

None of the covariates contained extremely large or small values, except for the covariate ‘reef cover’ for Anguilla and Saba Bank (Figure S4.1), which was subsequently converted into absence – presence (categorical) for Anguilla, and excluded from the model for Saba Bank, as it only contained 11 values > 0 out of a total of 172 transects. Variance inflation factors (VIF) and pairwise scatterplots indicated the presence of strong collinearity among covariates for all three sites, and the covariates ‘rubble cover’ and ‘seagrass cover’ were removed for all

models of the three sites (Table S4.1, Figure S4.2, S4.3, S4.4). The covariate ‘seagrass cover’ for Saba Bank was also removed because there were no values > 0 for this site.

The initial Poisson GLMs fitted were over-dispersed for all three sites, and the subsequent scatterplots of conch counts versus the covariates indicated some non-linear patterns. The covariates ‘sampling effort (Log)’ (total area [m²] of the transect) and ‘sand cover’ could be modeled as fixed effects (Equation 1, 2). Also, the covariate ‘reef cover’ could be modelled as a fixed effect: categorical for Anguilla and linear for St Eustatius. In contrast, non-linear patterns were found for the covariates ‘algae cover’, ‘depth’ and ‘distance to open ocean’ (DOO) for all three sites. These covariates were modelled as smoother functions. Initial GAMs, without spatial correlation, could not cope with the percentage of zeros in the data and were over-dispersed. Model validation showed that Pearson residuals were spatially correlated for all sites. The following GAMs with spatial correlation were therefore applied for the three locations: Anguilla (Equation 1), St Eustatius (Equation 1) and Saba Bank (Equation 2) (Zuur et al. 2018).

$$\begin{aligned}
 &Conch_i \sim Poisson(\mu_i) \\
 &E(Y_i) = \mu_i \quad \text{and} \quad var(Y_i) = \mu_i \\
 &\log(\mu_i) = \text{Intercept} + \text{LogSampling effort}_i + \text{Reef}_i + \text{Sand}_i + f_1(\text{Algae}_i) + \\
 &\quad + f_2(\text{Depth}_i) + f_3(\text{Distance open Ocean}_i) + u_i
 \end{aligned}$$

(Equation 1)

$$\begin{aligned}
 &Conch_i \sim Poisson(\mu_i) \\
 &E(Y_i) = \mu_i \quad \text{and} \quad var(Y_i) = \mu_i \\
 &\log(\mu_i) = \text{Intercept} + \text{LogSampling effort}_i + \text{Sand}_i + f_1(\text{Algae}_i) + \\
 &\quad + f_2(\text{Depth}_i) + f_3(\text{Distance open Ocean}_i) + u_i
 \end{aligned}$$

(Equation 2)

In Equation 1 and 2 the terms $f_1()$ to $f_3()$ are smoothing functions (splines) (Zuur et al. 2018), while the other terms are parametric effects. The term u_i in the model is a spatially correlated random intercept that is normally distributed with mean 0 and covariance matrix Ω .

For the three sites the models with spatial correlation produced lower WAIC values than the models without spatial correlation (Table 4.4). Model validation indicated that the spatial Poisson GAM + barrier for Anguilla and St Eustatius did not contain any remaining spatial correlation in the Pearson residuals, and that the models could cope with zero inflation. Similarly, the spatial Poisson GAM for Saba Bank did not contain any remaining spatial correlation in the Pearson residuals and the model could cope with zero inflation.

Table 4.4. Watanabe-Akaike information criterion (WAIC) for models with and without spatial dependence for the three survey sites for model selection. Variables used in the models are presented in Equations 1 and 2. * indicates the model selected.

Site	Model	WAIC
Anguilla	Poisson GAM with cr smoothers	565
	Spatial Poisson GAM with cr smoothers + barrier *	403
St Eustatius	Poisson GAM with cr smoothers	982
	Spatial Poisson GAM with cr smoothers + barrier *	622
Saba Bank	Poisson GAM with cr smoothers	1453
	Spatial Poisson GAM with cr smoothers *	746

4.3.2.2 Model inference

Anguilla

Algae cover was the only smoother with an important effect and demonstrated a decrease in conch numbers with algae cover, with higher amount of conch at low algae cover (0 - 0.45) and higher amounts of conch at higher algae cover (0.7 – 1.0) (Figure 4.3). Depth and DOO had no influence on conch abundance (Figure 4.3). Reef and sand cover both had important negative effects on the number of conch, while sampling effort (i.e. transect length) was not important (Table 4.4). The spatial random field showed two areas with relatively high values and one area with relatively low values of conch (Figure 4.2) and spatial correlation up to 1.2 km. Where the value of the spatial random field was around 2.5 (dark red areas), this indicated an $\exp(2.5) \approx 12.2$ times larger number of conch than the overall mean, due to spatial

dependency. Conversely, in the dark blue areas (values ca. -2), the model indicated conch values that were a factor $\exp(-2) \approx 7.3$ smaller than the overall mean. The posterior standard deviations were around 0.8 which means that w 's larger than 1.6 or smaller than -1.6 were deemed important (based on the normal distribution) (Figure S4.6).

St Eustatius

In St Eustatius, all smoothers were important (i.e. algae cover, DOO, and Depth). The algae cover smoother showed a bell-shaped pattern that caused lower values of conch at low algae cover (0 – 0.12) and higher values of conch at medium cover (0.25 – 0.62) (Figure 4.4). The depth smoother showed a similar bell-shaped pattern which caused lower values of conch between the 0 – 14 m interval and marginally higher values of conch in the 18 – 27 m interval. The DOO smoother showed lower values beyond 2.8 km (Figure 4.4). Sampling effort, sand and reef cover all had an important negative effect on conch counts (Table 4.4). The spatial random field (Figure 4.2) showed several areas with relatively high and relatively low values of conch, with a spatial correlation up to 1 km. When the spatial random field was around 3.5 (dark red areas), this indicated an $\exp(3.5) \approx 33$ times larger number of conch than the overall mean, due to spatial dependency. Conversely, in the dark blue areas (values ca. -2.5), the model indicated conch values that were a factor $\exp(-2.5) \approx 12.2$ smaller than the overall mean. Posterior standard deviations were around 1 which means that w 's larger than 2 or smaller than -2 can be deemed important (Figure S4.6).

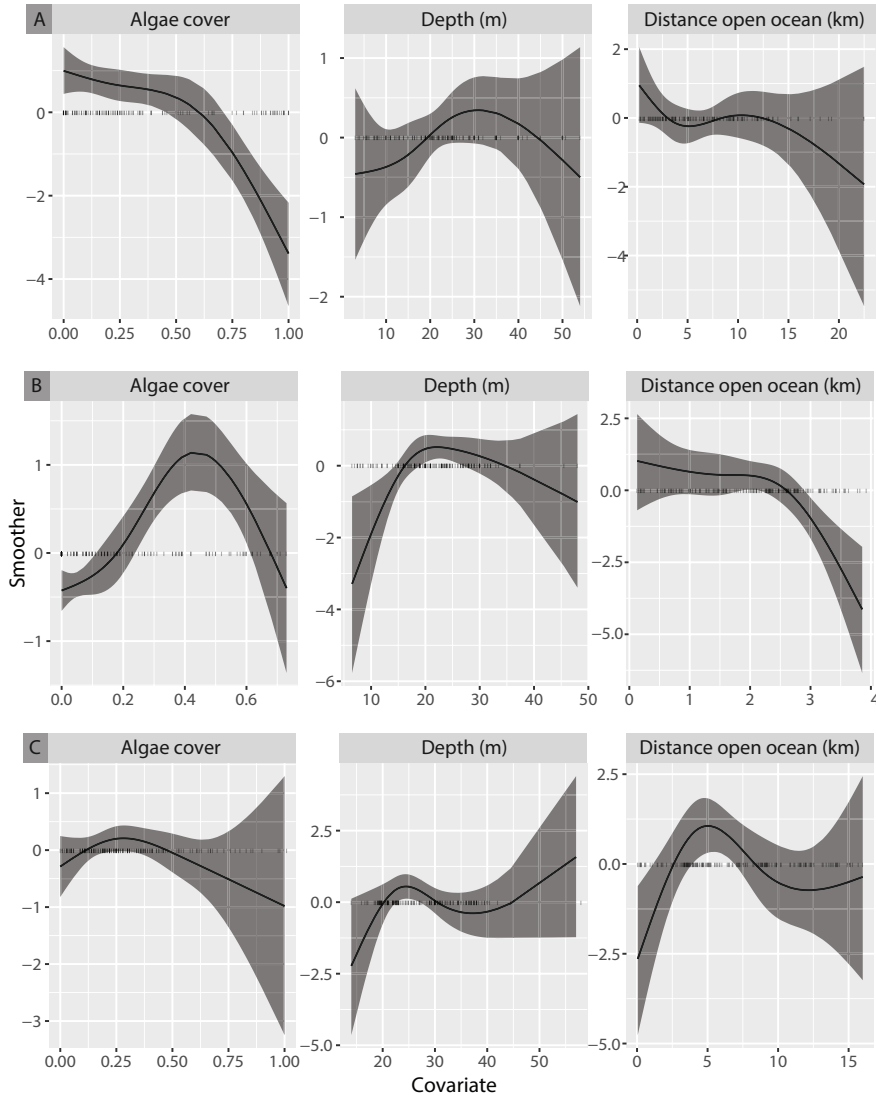


Figure 4.3. Marginal posterior effect of the relationship between conch counts and the 3 smoothers $f_1(\text{Algae})$, $f_2(\text{Depth})$, and $f_3(\text{Distance to Open Ocean})$ for Anguilla (A), St Eustatius (B), and Saba Bank (C) (mean and 95% credible interval). Due to the exponential link the exponential of each smoother is taken to obtain fitted values on the scale of the conch. The vertical 'I' symbols at $y = 0$ indicate where samples were taken.

Saba Bank

Depth was important and caused marginally higher values of conch between the 22 – 27 m interval (Figure 4.5). DOO was also important and caused lower values of conch in the 0 – 1 km interval and higher conch values between the 3.5 – 7 km interval (Figure 4.5). Algae cover had no influence on conch abundance (Figure 4.5). Neither of the two fixed covariates, sampling effort (i.e. transect length), nor sand cover, were important (Table 4.4). The spatial random field presented in Figure 4.2 showed several areas with relatively high (red areas) and relatively low (blue areas) values of conch with a spatial correlation up to 7 km. When the spatial random field was around 3.5 (dark red areas), this indicated an $\exp(2.5) \approx 12.2$ times larger number of conch than the overall mean, due to spatial dependency. Conversely, in the dark blue areas (values ca. -2), the model indicated conch values that were a factor $\exp(-2) \approx 7.3$ smaller than the overall mean. Posterior standard deviations were around 1 which means that w 's larger than 2 or smaller than -2 can be deemed important (Figure S4.6).

Table 4.5. Parametric effects of the spatial models for the three sites (Equation 1, 2). * indicates that the parametric effect is important. All parametric effects were modeled as linear except the covariate reef cover for *Anguilla* which was modeled as categorical (see section 2.5.1).

Anguilla	mean	Q_{0.025}	Q_{0.975}
Intercept	3.171	-1.332	7.706
Sampling effort (Log)	-0.270	-0.945	0.400
Reef cover *	-4.099	-5.964	-2.441
Sand cover*	-2.839	-3.962	-1.764

St Eustatius	mean	Q_{0.025}	Q_{0.975}
Intercept	5.1348	2.6216	7.7481
Sampling effort (Log)*	-0.7370	-1.1045	-0.3924
Reef cover*	-1.0111	-1.9368	-1.0274
Sand cover*	-1.1638	-1.9023	-0.4565

Saba Bank	mean	Q_{0.025}	Q_{0.975}
Intercept	3.1980	-8.9696	15.3139
Sampling effort (Log)	-0.4663	-2.3842	1.4548
Sand cover	0.2977	-0.6262	1.2189

4.4 Discussion

This study demonstrated distinct spatial distribution patterns of adult conch, which occurred in patchy distributions, with areas of high and low abundance of conch at all three sites. The patchy distribution of conch was caused by spatial dependency (Figure 4.2) and had a maximum magnitude of range up to 7 km for Saba Bank. The magnitude of range for spatial dependency for Anguilla and St Eustatius was smaller, up to 1.5 and 1 km respectively, which could have been (partly) caused by smaller survey areas and the presence of land barriers at these locations. The observed spatial dependency could represent either real dependency, originating from endogenous processes (population biological interactions) or exogenous processes (spatially structured environmental controls) (Planque et al. 2011), or other missing covariates, such as fishing pressure (Stoner et al. 2018). The spatial dependent models in this study did not capture the spatial dependency in conch, and was, therefore, in itself not related to habitat, geomorphological area, season, or any other covariate included in the model. This is in contrast to a previous study, where the spatial dependency in conch were captured by habitat covariates, when added to the model (Vallés & Oxenford 2012). However, our study did not support this conclusion and suggests that spatial dependency is more likely to originate from endogenous processes, such as aggregating behavior, rather than exogenous processes (spatially structured environmental controls) (Planque et al. 2011). Endogenous processes in conch which influence conch to aggregate have been identified. Stoner & Ray-Culp (2000) demonstrated an Allee effect in conch where reproductive activity begins to decline as densities of conch decrease below approximately 200 conch/ha, consequently, conch tend to aggregate, which can at least partially explain the spatial dependency observed in conch abundance during the reproductive season (approximately 50 % of transects were conducted during the reproductive season). However, due to their limited home range (ca. < 1 – 6.5 ha) and daily movement patterns (ca. 11 m/day) (Glazer et al. 2003, Delgado & Glazer 2007, Doer & Hill 2013, Stieglitz & Dujon 2017) is it likely that such aggregation persists outside the breeding season. Nevertheless, currently undetermined missing factors cannot be ruled out, and the discovery and addition of such factors could potentially shed further light on the cause of spatial dependency in adult conch.

The Bayesian hierarchical spatial models for St Eustatius also showed fewer conch in shallow depths (0-15 m) and a small increase in conch abundance just below 20 m for St Eustatius and Saba Bank (Figure 4.3). Environmental factors likely influenced the lack of conch in shallow depths (0-15 m) at the three locations. Fishing has been known to change the depth distribution of conch and can shift conch distributions to greater depths depending on the methods used (Stoner & Schwarte 1994). Although Saba Bank has had a complete moratorium on the conch fishery since 1994 (Hoetjes & Carpenter 2010), and can thus be expected to have conch population close to its natural distribution, it has a shallowest point of ca. 15 m. Thus, the conch population has a deeper natural distribution in comparison to published literature (Weil & Laughlin 1984, Ehrhardt & Valle-Esquivel 2008). Conch are caught in St Eustatius, although at relatively low amounts (3 % of the total adult population) (Meijer zu Schlochtern 2014). However, the lack of high densities of conch at shallow depths was likely due to shallow habitats being either unsuitable (high relief areas with corals reef) (Debrot et al. 2014) and/or exposed to the elements which deter conch from settling in shallow areas around the island. Contrary to St Eustatius, shallow areas around Anguilla were often more sheltered and the lack of high abundance of conch in these seemingly suitable areas is likely to be at least partially driven by fishing pressure, which was approximately 6% of the total adult population (Kuramae Izioka 2016). Slightly lower than the 8 % recommended by the Queen Conch Expert Workshop Group Report (2012).

In contrast to shallow waters, high densities of conch (> 250 /ha) were found in waters > 30 m at all three survey sites. Although conch are known to be most common in depths < 25 m (Ehrhardt & Valle-Esquivel 2008), high densities (ca. 300/ha) of adult conch during the peak reproductive season have been found in deep water habitats (Garcia-Sais et al. 2012). Similarly, the current study found densities of > 300 adult conch/ha at Saba Bank at a depth of 40 m both during and outside the peak reproductive season (Boman et al. 2018). This indicates that at all three sites, deep water (ca. 17 – 46 m) conch comprise the majority of reproductive output and are highly important for reproductive success and capacity in these areas. Furthermore, these results highlight the importance of surveying areas beyond the practical depth limitation of divers (Queen Conch Expert Workshop Group Report 2012) to fully estimate of the entire depth range of conch and obtain more reliable population estimates.

Substrate-associated covariates (Table 4.3) from the Bayesian hierarchical spatial models for the three sites did not reveal consistent patterns explaining the distribution of conch. However, reef cover consistently had an important negative effect on conch numbers. This resulted from the Bayesian models for Anguilla and St Eustatius, and although reef cover could not be included in the Bayesian model for Saba Bank, conch were completely absent in areas with high-reef substrate cover. This is consistent with previous studies which indicate that conch are not commonly found in high-relief areas, including areas with high reef cover (Acosta 2006). Sand cover had an important negative effect on conch abundance (Table 4.4), which contradicts the general notion of sand habitats being recognized as suitable conch substrates (Torres-Rosado 1987, Acosta 2001, Stoner & Davis 2010). In this study, high abundances (> 250 / ha) of conch were also present in areas with high sand cover (> 90 %). Therefore, it is unlikely that there is a general negative effect of increased sand cover on conch distribution. Instead, within suitable substrates (e.g. sand, rubble) the abundance and distribution of conch is more likely to be influenced by other factors such as depth and algae cover, as well as the natural patchy distribution of conch.

In the current study, high levels of algae cover were mostly associated with low conch numbers. However, algae cover included all macroalgae and cyanobacteria mats, while further in-depth analysis to species level was not performed. As the majority of the algae species in the surveys were calcified or possessed chemical defenses (e.g. *Halimeda* spp, *Dictyota* spp, *Caulerpa* spp), these are unpalatable to most marine species and unlikely to be a significant source of nutrition for conch (Hay et al. 1987, Pereira et al. 2002, Erickson et al. 2006, Pereira & de Gama 2008). Associations between queen conch abundances and macroalgae cover were thus not likely based on foraging behavior. Whilst the results from this study (Figure 4.3) indicate a maximum threshold for algae cover and possibly an optimal level of macroalgae cover for conch, we hypothesize that macroalgae cover to a certain level may be beneficial for adult conch due to a potential increased in available food sources (macrophyte epiphytes). However, when algae cover reaches a certain level (ca. 0.6-0.7), other sources of nutrition (e.g. benthic diatoms) may be reduced by light competition with macroalgae (Hill 1996, Yang & Flower 2012), which cannot be fully compensated for by the potential epiphytic food source on the macroalgae.

Bayesian hierarchical spatial models demonstrated that DOO has an important effect on conch abundance on Saba Bank. Fewer conch were observed close to the edge of the bank (0 - 1 km), and higher abundances of conch occurred just inside this area (3.75 – 7 km) (Figure 4.5). However, the model's prediction of few conch close to the edge was likely caused by a series of transects located on the shallow eastern edge of the bank, which is an actively growing coral reef zone (Van der Land 1977). No conch were found in this reef zone, which is unsurprising given that conch are not usually found in coral reef habitats (Acosta 2006). As reef cover was not included as a covariate in the model for Saba Bank, this factor could not provide an explanatory effect on the low amount of conch in this habitat shown by the DOO covariate. Tidal current flow has been found to govern the distribution of conch in The Bahamas, where adult conch were positively associated with tidal channels with high tidal flow and were found in higher abundances closer to the tidal channel (Kough et al. 2017). Offshore banks (e.g., Saba Bank) have a tidal current flow which is strongest at the edge of the bank, closer to the open ocean, and weaker in the center of the bank (Hamner & Hauri 1981). The higher abundance of conch found in this study toward the edge of Saba Bank is thus likely an effect of tidal flow on the distribution of conch, and supports previous suggestions of an ecological importance of water-flow for conch and their distribution patterns (Kough et al. 2017). Small islands with a narrow shelf, such as Anguilla and St Eustatius, have a more complex water flow pattern that is not directly related to distance to ocean and is dependent on a range of factors (e.g. shape and size of island mass, direction of water flow, topography of area around the island), creating areas of high and low water flow downstream of the island (Hamner & Hauri 1981). Therefore, as seen in Anguilla and St Eustatius, conch abundance is not expected to concur with distance to open ocean, and more complex measurements of water flow patterns will likely be necessary to elucidate whether abundance and distribution of conch are influenced by areas of high and low water flow at such locations.

Spatial dependency and a patchy distribution of conch, independent of the abiotic and biotic factors tested (e.g. substrate, macrophyte cover, distance to ocean), appears to be generally applicable to conch populations as this pattern was visible in areas with different geomorphology, size, and habitat homogeneity. The lack of strong generic location over-crossing relationships between abiotic and biotic factors and adult conch abundance and distribution is likely partly due to this spatial dependency, as well as different location-specific

factors (e.g., patterns of water flow) that affect different aspects of conch life-history. Furthermore, conch are often unlikely to position themselves optimally in accordance to important factors due to a trade-off situation, which was observed on Saba Bank where unsuitable habitat (high relief reef) likely prevented conch to move to the further edge of the bank where the highest water flow is found.

Although substrate and depth can, to a certain extent, predict conch abundance and distribution patterns, specific percentage cover of suitable substrate (i.e. sand, rubble) and specific depth in the approximate range 0 - 45 m are not reliable factors for predicting adult conch abundance and distribution patterns. The results from the current study demonstrated high densities of conch (> 300/ha) at depths > 30 m, and indicate that the current notion of a most common depth range of conch (< 25 m) (Weil & Laughlin 1984, Ehrhardt & Valle-Esquivel 2008) may need revision, whereby deeper areas should not be excluded from conch surveys when present. This general notion of a preferred depth range could in part be due to conventional survey methods being impractical for surveys beyond this depth range and thus are biased towards deeper areas (> 25 m). The notion could also in some areas be a by-product of long-term over-exploitation that has restricted the remaining population to less-accessible, deeper habitats (Stoner et al. 2018).

Another factor that can complicate the prediction of natural abundance and distribution patterns of conch is fishing pressure, which most visibly disrupts the natural depth-distribution patterns of conch when shallow, more easily caught conch are usually targeted first. It is, however, unlikely to have influenced the relationships between abiotic and biotic factors and conch abundance and distribution in the current study to a high degree. The general harvesting of conch can have a dampening effect on some relationships between conch and different factors due to an overall decrease in conch abundance that makes natural distribution patterns more difficult to detect.

It is important to acknowledge that even though the same factors are likely to influence conch distribution patterns in every location due to local conditions (e.g., geomorphology, waterflow patterns, habitat homogeneity, depth, macrophyte cover), these factors will not shape distribution patterns in exact the same way in all locations. Consequently, it is not a one-size-fits-all situation. Modelling and prediction of conch abundance and distribution patterns

needs to consider not only local conditions but also spatial dependency and variables, such as macroalgae cover, in addition to common variables such as general habitat and depth.

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Supplementary Material

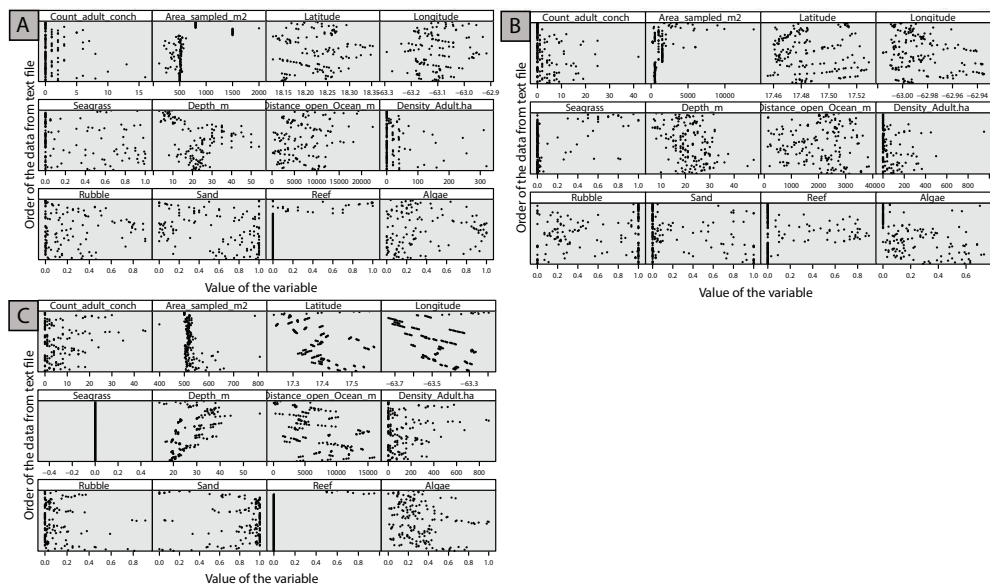


Figure S4.1. Cleveland dots plots for Anguilla (A), St Eustatius (B) and Saba Bank (C).

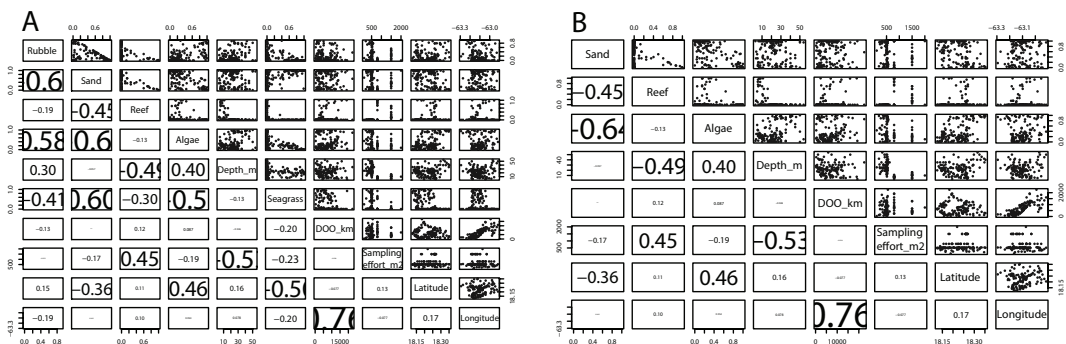


Figure S4.2. Pairwise scatterplots of all initial covariates (A) and covariates of the final model (B) for Anguilla.

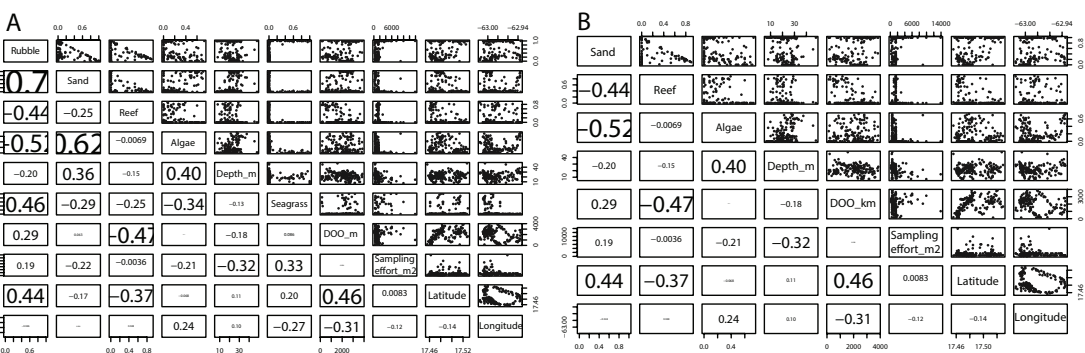


Figure S4.3 Pairwise scatterplots of all initial covariates (A) and covariates of the final model (B) for St Eustatius.

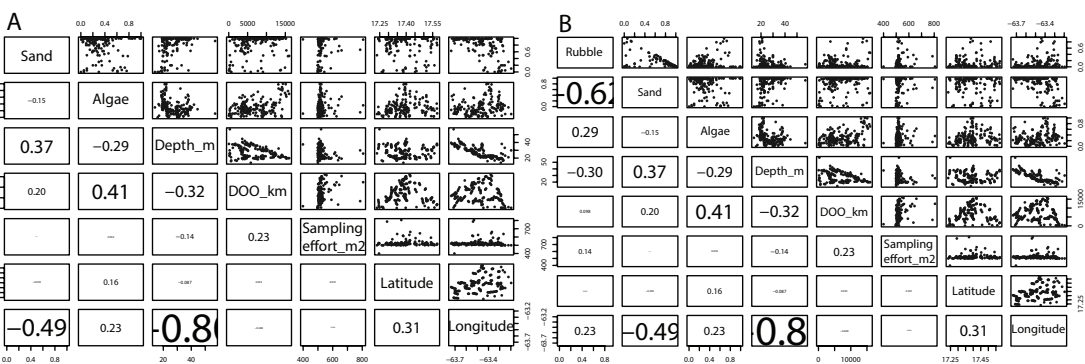


Figure S4.4 Pairwise scatterplots of all initial covariates (A) and covariates of the final model (B) for Saba Bank.

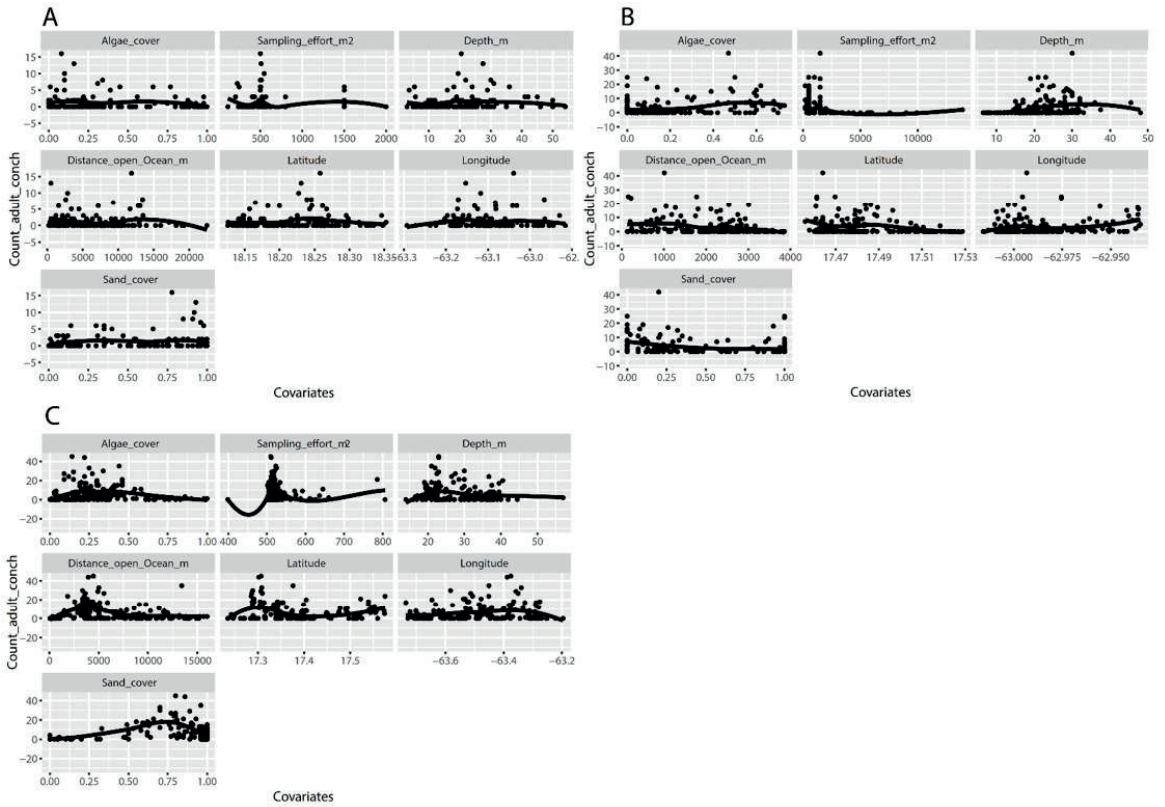


Figure S4.5. Scatterplots of conch counts versus all covariates in the final model for Anguilla (A), St Eustatius (B), and Saba Bank (C).

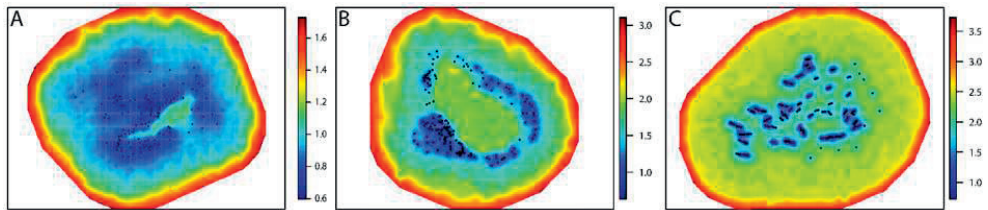


Figure S4.6. Standard deviation of the spatial random field for Anguilla (A), St Eustatius (B) and Saba Bank (C). Standard deviations of the spatial random field are around 0.8, 1.0 and 1.0 in the area at the sampling locations (conducted transects = black dots) for Anguilla, St Eustatius and Saba Bank respectively. This means that the value of the spatial random field (Figure 4.2) that is smaller than - 1.6, -2.0, and -2.0 and larger than 1.6, 2.0, and 2.0 for Anguilla, St Eustatius and Saba Bank respectively, indicated important effects.

Table S4.1. Variance inflation factors (VIF) values for Anguilla, St Eustatius and Saba Bank.

Anguilla		St Eustatius		Saba Bank	
Variance inflation factors (VIF)		Variance inflation factors (VIF)		Variance inflation factors (VIF)	
Rubble_cover	3.191903	Rubble_cover	9.701031	Rubble_cover	1.974089
Sand_cover	6.471144	Sand_cover	10.848469	Sand_cover	2.368782
Reef_cover	4.323219	Reef_cover	5.434494	Algae_cover	1.408758
Algae_cover	4.200887	Algae_cover	2.039813	Depth_m	5.247715
Seagrass_cover	2.569377	Seagrass_cover	1.564676	Distance_open_Ocean_m	2.17423
Depth_m	2.04302	Depth_m	1.611402	Sampling_effort_m2	1.09054
Distance_open_Ocean_m	3.256273	Distance_open_Ocean_m	2.013958	Latitude	1.33465
Sampling_effort_m2	1.860434	Sampling_effort_m2	1.24439	Longitude	5.580976
Latitude	1.94423	Latitude	1.624112		
Longitude	3.164051	Longitude	1.385402		
Variance inflation factors (VIF)		Variance inflation factors (VIF)		Variance inflation factors (VIF)	
Sand_cover	3.788459	Sand_cover	2.31268	Sand_cover	1.422978
Reef_cover	2.773084	Reef_cover	1.737922	Algae_cover	1.352142
Algae_cover	3.782286	Algae_cover	1.902255	Depth_m	5.122584
Depth_m	1.947451	Depth_m	1.594929	Distance_open_Ocean_m	2.170986
Distance_open_Ocean_m	3.186229	Distance_open_Ocean_m	1.907117	Sampling_effort_m2	1.066393
Sampling_effort_m2	1.667882	Sampling_effort_m2	1.159357	Latitude	1.330145
Latitude	1.795059	Latitude	1.617602	Longitude	5.314442
Longitude	3.131662	Longitude	1.271392		

Priors and mesh size

We used the default priors and hyperparameters currently implemented in R-INLA. For further information, refer to R-INLA documentation available on the website (<http://www.r-inla.org>). We also used penalized complexity priors (PC-priors) to construct a Matérn SPDE model, characterized by spatial correlation range r and standard deviation parameter σ , with probability $P(r < 3 \text{ km}) = 0.0001$ and $P(r > 0.5) = 0.0001$ for Anguilla, $P(r < 1 \text{ km}) = 0.05$ and $P(r > 0.5) = 0.05$ for St Eustatius, $P(r < 15 \text{ km}) = 0.01$, and $P(r > 0.5) = 0.5$ for Saba Bank. In the current spatial models for the three locations Anguilla, St Eustatius, and Saba Bank, the mesh sizes were 3,600, 5,753, and 7,892 respectively.

Chapter 5

Diet and growth of juvenile Queen conch *Lobatus gigas* (Gastropoda: Strombidae) in native, mixed and invasive seagrass habitats.

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Marine Ecology Progress Series, 621: 143-154

Abstract

Juvenile queen conch are primarily associated with native seagrass such as *Thalassia testudinum* in large parts of their range in the Caribbean and the southern Gulf of Mexico. Here, a number of non-native seagrass species have been introduced including *Halophila stipulacea*, which is natural to the Red Sea and the Indo-Pacific. In the Caribbean, *H. stipulacea* often creates dense continuous mats with little or no sediment exposed, compared to native seagrass which grows much less dense. We examined the diet and growth of juvenile conch in both native, mixed, and invasive seagrass beds using stable isotope analysis and an *in situ* growth enclosure experiment. Organic material in the sediment (i.e. benthic diatoms and particulate organic matter) was found to be the most important source of carbon and nitrogen for juvenile queen conch in all three habitats investigated, and there was a significantly higher probability of positive growth in the native seagrass compared to the invasive seagrass. Due to the importance of the organic material in the sediment as a source of nutrition for juvenile conch, limited access to the sediment in the invasive seagrass can potentially cause inadequate nutritional conditions to sustain high growth rates. Thus, it is likely that there is a negative effect on juvenile queen conch growth currently inhabiting invasive seagrass beds, compared to native seagrass beds, when other potential sources of nutrition are not available.

5.1. Introduction

Lobatus gigas, commonly called queen conch (Gastropoda: Strombidae), is an economically and culturally important marine gastropod (Brownell & Stevely 1981, Appeldoorn 1994) found throughout the Wider Caribbean Region and the southern Gulf of Mexico. The species is heavily exploited throughout large parts of its natural range (Stoner 1997) which has led to a decrease in population densities, a truncation of size classes (Stoner et al. 2012b) across much of the species' distribution range (Acosta 2006), and even towards overfishing (Stoner et al. 2018). Due to concerns for the continued over-exploitation of the species (Stoner et al. 2012b), measures have been taken such as the listing of the species to Appendix II of the Convention on International Trade in Endangered Species of Fauna and Flora (CITES) in 1992.

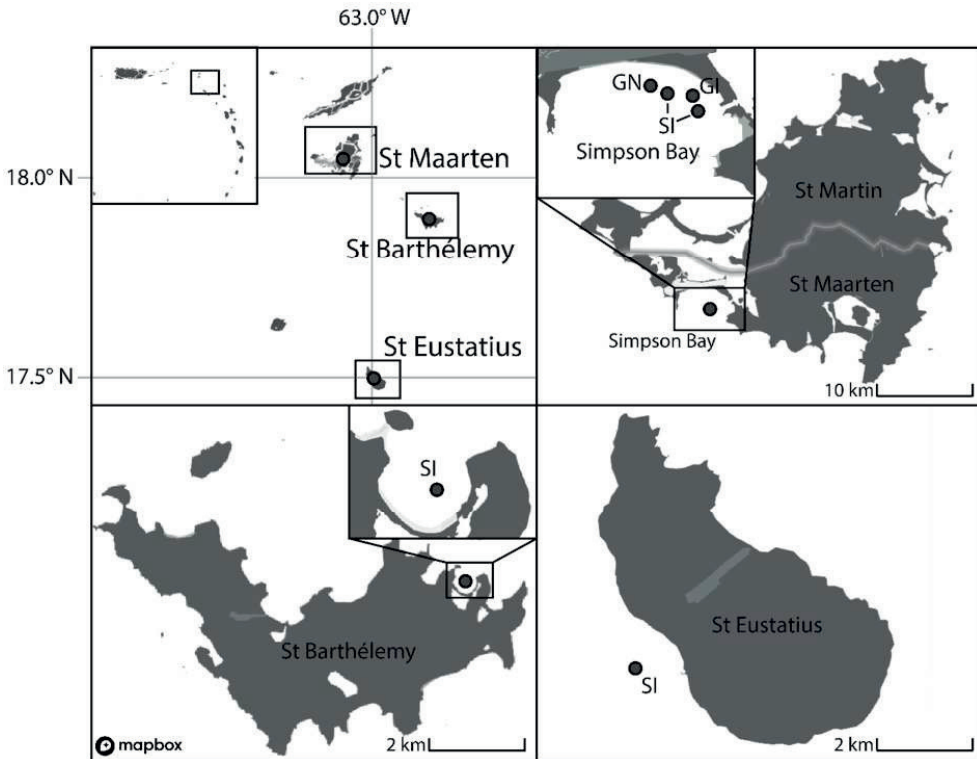


Figure 5.1. Map showing the locations for the growth experiments and stable isotope analysis collection areas in St Barthélemy (native site), St Eustatius (invasive site) and St Marten (mixed site) in the eastern Caribbean. SI = Stable isotope sampling location, GN = growth experiment in native seagrass (*T. testudinum* and *S. filiforme*) and GI = growth experiment in invasive seagrass (*H. stipulacea*).

Adult conch can be found in a wide range of environmental conditions (Stoner et al. 1994) such as sand and algal or coral rubble (Acosta 2001, Stoner & Davis 2010). Juvenile conch, on the other hand, appear to have more specific habitat requirements (Stoner et al. 1994), and in large parts of their distribution range (e.g. The Bahamas) juvenile conch are associated primarily with native seagrass such as *Thalassia testudinum* (Stoner 2003), which provides both nutrition and protection from predators (Ray & Stoner 1995, Stoner 2003, Stoner & Davis 2010). The primary diet of juvenile conch consists of native seagrass detritus, and red and green macroalgae, primarily *Laurencia* spp. and *Batophora oerstedii* (Randall 1964, Stoner & Sandt 1991, Stoner & Waite 1991, Serviere-Zaragosa et al. 2009). The production of red and green algae, which can be highly variable, has been shown to directly affect the

growth of juvenile conch (Stoner et al. 1994, Stoner et al. 1995, Stoner 2003). Organic material in the sediment (benthic diatoms and particulate organic matter [POM]) and cyanobacteria have also been suggested to be sources of nutrition to juvenile conch (Stoner & Waite 1991, Stoner et al. 1995, Serviere-Zaragosa et al. 2009).

Different species of seagrass vary in leaf width, leaf structure, canopy height, and leaf lifespan (Trautman & Borowitzka 1999, Horinouchi et al. 2009). As a result, individual seagrass species host unique epiphytic communities, which form different food sources, and provide shelter to a variable degree, depending on their morphological characteristics (Trautman & Borowitzka 1999, Horinouchi et al. 2009). Consequently, seagrass composition determines the associated species assemblages (Bologna & Heck 1999, Willette & Ambrose 2012, Olinger et al. 2017). Alterations to the composition of seagrass beds, such as through the introduction of invasive species, can thus cause changes in the associated biota and change the level of shelter and the quality and quantity of food sources (Willette & Ambrose 2012).

In the Caribbean, a number of such non-native species have been introduced (Kairo et al. 2003) including the seagrass *Halophila stipulacea* (Williams 2007), which is natural to the Red Sea and the Indo-Pacific. *H. stipulacea* has, since its first observation in 2002 on Grenada (Ruiz & Ballantine 2004), spread rapidly and can now be found around at least 19 islands throughout the eastern Caribbean and the northern parts of South America (Vera et al. 2014, Willette et al. 2014). *H. stipulacea* can displace native seagrass species such as *Syringodium filiforme*, *Halodule wrightii*, and *Halophila decipiens* (Willette & Ambrose 2012). It is able to spread at a high rate, with an observed expansion from 316 ha to 773 ha in Dominica during a five-year period (Steiner & Willette 2013). Knowledge of the impact of *H. stipulacea* on the local fauna is limited, although reductions in the relative number of small and juvenile fishes have been reported (Willette & Ambrose 2012, Olinger et al. 2017). The effects of *H. stipulacea* on the habitat quality for juvenile queen conch are not clear. It can be expected that the presence and abundance of certain food items may change due to changed seagrass composition, which could translate in diet shifts and eventually in altered growth rates (Stoner et al. 1994, Stoner et al. 1995, Stoner 2003). Direct examination of stomach contents to determine diets is problematic and only provides an estimate of recently ingested items and limited information on the degree of assimilation of dietary

items (Garcia et al. 2007, Pasquaud et al. 2008). These limitations have promoted the use of stable isotope analysis and isotopic mixing models, which have the major advantage that they can provide information on the direct assimilation of dietary items by the consumer (Fry 2006, Bond & Diamond 2011, Parnell et al. 2013). Interpretation of stable isotope ratios is generally based on the assumption that with each trophic level there is a constant enrichment in the heavier isotopes due to the discrimination towards lighter isotopes during metabolism and excretion (DeNiro & Epstein 1981, Peterson & Fry 1987). Enrichment of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ differs between habitats and taxa, with mollusks, in general, having a low $\delta^{15}\text{N}$ enrichment of approximately 1 - 1.5 ‰ between diet and consumer (Vanderklift & Ponsard 2003). Gastropods have also been found to have $\delta^{15}\text{N}$ isotopic values reflecting the exposure of sites to anthropogenic impact (Vermeulen et al. 2011), with higher $\delta^{15}\text{N}$ isotopic values at sites with higher $\delta^{15}\text{N}$ baseline (Vermeulen et al. 2011). This gives the possibility to use stable isotope analysis not only for diet investigation but also for assessing nitrogen pollution (Bannon & Roman 2008).

The purpose of this study is twofold. First it examines the diet of juvenile conch in a variety of native, invasive, and mixed native / invasive seagrass beds using stable isotope analysis. Isotopic values of conch are expected to vary in accordance with their diet but also to factors such as the baseline $\delta^{15}\text{N}$ at the different sites, which may include different levels of anthropogenic disturbance. In addition, the contribution of *H. stipulacea* detritus to the diet of juvenile conch is explored.

Secondly, this study examines juvenile queen conch growth in an *in situ* enclosure experiment comparing growth rates of juveniles in native, invasive and mixed native / invasive seagrass beds. Growth is expected to be better in habitats which provide better and more abundant access to food sources of the proper nutritional quality. Both the dietary study and the growth experiment will be used to discuss the potential consequences of the *H. stipulacea* invasion on the development of queen conch populations.

5.2 Material and Methods

5.2.1 Study area

The study was performed in seagrass beds at three geographic locations in the eastern Caribbean (St Barthélemy, St Eustatius, and St Maarten; Figure 5.1). Samples for dietary analysis were collected at all three locations, from sites with sandy bottoms, located at 1–20 m depth, 20–900 m from shore, with 20–95% seagrass cover, and with <1% macroalgae cover. Seagrass species composition differed between sites (St Barthélemy: native, St Eustatius: invasive, and St Maarten: mixed native / invasive) (Table 5.1). Only native seagrass species were found at the native site, while both native and invasive seagrass species were found at the mixed and invasive site. However, only a very small proportion (<1% of total cover) of native seagrass (i.e. *Syringodium filiforme*) were found at the invasive site, while the proportion of native and invasive seagrass species at the mixed site were roughly equal (Table 5.1). The growth experiment was conducted at two sites in Simpson Bay, St Maarten (Figure 5.1), which differed in seagrass composition (i.e. native vs. invasive), while all other relevant environmental variables were similar. Both sites had sandy substrates, were located at 4–5 m depth, were 250–270 m from shore, had 40–95% seagrass cover, and <1% macroalgae cover. Variations in water temperature, light availability and nutrient concentrations were considered negligible in view of the proximity (~500 m) of both locations towards each other (Table 5.2).

Table 5.1. Stable isotope collection site (STB = St Barthelemy, STM = St Maarten, STE = St Eustatius) conditions (seagrass habitat, substrate, conch shell length [SL], depth, distance to shore, total area of seagrass and macroalgae covering the substrate, and relative cover of each individual seagrass species [*H.s* = *H. stipulacea*, *T.t* = *T. testudinum*, *S.f* = *S. filiforme* and *H.w* = *H. wrightii*]).

Site	Seagrass Habitat	Substrate	Conch SL (mm)	Depth (m)	Distance shore (m)	Total cover (%)		Relative cover total (%)			
						Seagrass	Macro Algae	<i>H. s</i>	<i>T. t</i>	<i>S. f</i>	<i>H. w</i>
STB	Native	Sand	106 - 206	1 - 3	20 - 200	30 - 60	< 1	0	35	35	30
STM	Mixed	Sand	112 - 160	4 - 6	200 - 300	20 - > 95	< 1	35	35	30	0
STM	Mixed	Sand	115 - 203	4 - 6	200 - 300	20 - > 95	< 1	30	35	35	0
STE	Invasive	Sand	106 - 215	14 - 20	400 - 900	20 - > 95	< 1	99	0	1	0

Table 5.2. Growth experiment site conditions (seagrass habitat, substrate, conch shell length [SL], depth, distance to shore, total area of seagrass and macroalgae covering the substrate [Total cover (%)], and seagrass shoot density [shoot/m²] of *H.s* = *H. stipulacea*, *T.t* = *T. testudinum* and *S.f* = *S. filiforme*) of the 6 enclosures (E) at St Maarten.

E #	Seagrass Habitat	Substrate	Conch SL (mm)	Depth (m)	Distance shore (m)	Total cover (%)		Seagrass shoot/m ²		
						Seagrass	Algae	<i>H. s</i>	<i>T. t</i>	<i>S. f</i>
1	Native	Sand	120 - 136	5	270	45	<1	0	842	640
2	Native	Sand	120 - 139	5	270	45	<1	0	821	608
3	Native	Sand	120 - 136	5	270	40	<1	0	741	618
4	Invasive	Sand	120 - 138	4	250	> 95	<1	3493	0	0
5	Invasive	Sand	121 - 139	4	250	> 95	<1	3701	0	0
6	Invasive	Sand	120 - 135	4	250	> 95	<1	3482	0	0

5.2.2 Diet analysis

5.2.2.1 Sample collection, preparation, and stable isotope analysis

Juvenile conch, which could be identified by the absence of a developed lip (Stoner et al. 2012a, Boman et al. 2018), ranging from 106 – 215 mm shell length, were collected by hand for dietary analysis from each of the three collection sites. A mantle tissue sample of approximately 0.5 x 0.5 cm x thickness of mantle (ca. 0.5 cm) was taken from each collected conch, before returning it live to the collection site. In addition, at each site, between 1 and 24 food items, previously indicated to be a source of conch nutrition (Randall 1964, Stoner & Sandt 1991, Stoner & Waite 1991, Serviere-Zaragosa et al. 2009), were collected by hand in close proximity to the conch (Table 5.3). The upper 3 cm of the surface sediment was also collected, using a 4 cm diameter core sampler (as sediment was expected to contain diatoms as potential food source), as well as seagrass detritus, and seagrass epiphytes, whenever present. Seagrass detritus was defined as dead leaves (no traces of green) that started to breakdown, but were still visually recognizable to belong to a certain species. Leaves with heavy epiphytic growth were collected and the epiphytes on the leaves were later carefully

scraped off with a scalpel in the lab. Sampling in St Eustatius was done between 10 August and 11 October 2017, both before and after hurricane Irma (6 September 2017), which had no perceivable impact at this location. Sampling in St Maarten took place between 29 and 31 of August 2017, and sampling in St Barthélemy was done between 9 and 11 January 2018.

The samples were stored in plastic bags in a cooler at ca. 4°C, while transported to the laboratory, where they were dried overnight at 60–70 °C and subsequently homogenized using a mortar and pestle. All sediment samples were treated with phosphoric acid to remove excess calcium carbonate, leaving the organic material in the sediment, most likely consisting of benthic diatoms and POM, which are common in marine sediment (MacIntyre 1996, Mann 1999, Jones et al. 2014). All other categories of food items were tested for inorganic content and were also treated with phosphoric acid if necessary. Between 0.5–60 mg of powdered sample was analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, using a Flash 2000 elemental analyzer coupled to an isotope ratio monitoring mass spectrometer (EA-irMS) via an Isolink IV (Thermo Scientific, Bremen, Germany), at the stable isotope laboratory at the Royal Netherlands Institute for Sea Research (NIOZ) at Texel, the Netherlands. As the C:N ratio of the conch mantle tissue was <3.8, with the majority of the samples (81 %) having a value <3.5, there was no need for a lipid correction (Post et al. 2007).

5.2.2.2 Statistical analysis of the diet data

A Kruskal-Wallis rank sum test was used to analyze differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among the detritus from seagrass species at each site separately, as well as for the three cyanobacteria mats found in St Eustatius. In case of significant overall differences, pairwise comparisons were applied, using the `kruskalmc` function from the package 1 (Giraudoux, 2018) in the R environment (R Core team 2017).

The stable isotope mixing model package (`simmr`), using a Bayesian statistical framework (Parnell 2016) was used to estimate proportional contributions of potential food sources to the diet of juvenile conch. `simmr` was implemented with 100 000 iterations (discarding the first 10 000). The estimated proportions of food sources in the diet mixture of juvenile conch were subsequently determined using a Markov Chain Monte Carlo function to repeatedly estimate the proportions of the various sources in the mixture and determine the values

which best fit the mixture data (Parnell 2016). The median (50% quantile), lower (25%) and upper (75%) quartiles, and 95 % credibility interval (2.5% and 97.5% quantiles) of the contribution of each of the food sources were determined.

Table 5.3. Items and number of items collected and analyzed at the three collection sites for dietary analysis.

	St Barthélemy	St Eustatius	St Maarten
Conch	24	25	23
Cyanobacteria (Red)		14	
Cyanobacteria (Brown A)		5	
Cyanobacteria (Brown B)		7	
Sediment	14	16	10
<i>H. stipulacea</i> Detritus		24	22
<i>H. stipulacea</i> Epiphytes		1	
<i>S. filiforme</i> Detritus	7	3	17
<i>H. wrightii</i> Detritus	6		
<i>T. testudinum</i> Detritus	18		15
<i>T. testudinum</i> Epiphytes	5		

5.2.3 Growth experiment

5.2.3.1 Conch collection and experimental set-up

Growth experiments were performed at two sites in Simpson Bay, St Maarten (i.e. Simpson Bay Airport and Simpson Bay Bridge) (Figure 5.1). In total 60 juvenile conch of shell lengths between 120 and 139 mm were collected by hand on 5 and 6 July 2017, and distributed over 3 enclosures at each site. Enclosures (4.5 x 4.5 m) consisted of ridged plastic netting approximately 30 cm high, held in place with steel bars driven into the sand. Ten conch were placed in each enclosure, with three enclosures located in the invasive seagrass *H. stipulacea* and three in a mix of native seagrass (*T. testudinum* and *S. filiforme*) (Table 5.2). Each conch was fitted with a small metal tag and a unique number to be able to recognize each individual. Initial shell length of each individual was measured as well as a final shell measurement at the end of the experimental period. No conch went missing or was found

dead inside the enclosures during the length of the experiment. Conch density (0.5 conch m^{-2}) in the enclosures was thus kept constant throughout the experiment. Seagrass shoot density and macroalgae cover was counted before the experiment within three quadrates ($25 \times 25 \text{ cm}$) randomly placed inside each enclosure (Table 5.2). Mean seagrass shoot densities at the native seagrass beds ranged between 608 and 640 shoots m^{-2} for *T. testudinum* and between 741 and 842 shoots m^{-2} for *S. filiforme*, while seagrass shoot density of invasive *H. stipulacea* ranged between 3482 and 3701 shoots m^{-2} . Macroalgae in the enclosures consisted entirely of calcified macroalgae species (i.e. *Dictyota* sp, *Caulerpa* spp, *Udotea* spp, *Halimeda* spp, *Penicillus* spp). The intent was to run the growth experiment for a total of 16 weeks, but due to a direct hit of a major hurricane (Irma), the growth experiment already ended after 47 days, because all macrophyte cover, much of the substrate, all conch, and all enclosure structures were removed by the hurricane. Also, a count of seagrass shoots after the experiment and grain size estimation of the substrate was not possible due to these circumstances. Yet, it was possible to collect data on conch growth based on 47 days (6.5 weeks) of observation.

5.2.3.2 Statistical Analysis of the growth experiment

Growth rates (shell length growth in mm day^{-1}) were calculated for each individual for the entire experimental period (6.5 weeks), and subsequently assigned to growth rate bins of 0.05 mm day^{-1} , ranging from -0.10 to $+0.15 \text{ mm day}^{-1}$. The probability of a positive growth rate was modelled as a binomial generalized linear model (GLM) with a logit function, with habitat (categorical covariate with two levels: native and invasive) as a fixed covariate. A binomial model with enclosure as random intercept was also fitted but the simpler model without the random effect had a lower AIC value and was thus chosen as the final model. Statistical analysis was conducted using the packages *car* (Fox & Sanford-Weisberg 2011), *lme4* (Bates et al. 2015), and *rmisc* (Hope 2013), in the R software environment (R Core Team 2017).

5.3 Results

5.3.1.1 Stable isotope profiles

Juvenile conch

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values varied between and within sampling sites (Figures 5.2, 5.3), but in the different habitats juvenile conch had a similar position in the iso-space plot in relation to the potential food sources (Figure 5.2). Juvenile conch in St Eustatius did, however, have more depleted $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than the conch in St Maarten and in St Barthélemy (Figure 5.3). Organic matter in the sediment was the only sampled food source which consistently had values of $\delta^{15}\text{N}$ which were similar or more depleted than the conch and with $\delta^{13}\text{C}$ values consistently more depleted than the conch at each site (Figure 5.2).

Seagrass detritus

The $\delta^{13}\text{C}$ detrital values from the native and invasive seagrass species were in general more enriched compared to conch with only a few values of *T. testudinum* and *H. wrightii* in St Barthélemy (native) which overlapped with conch at that site (Figure 5.2). The $\delta^{15}\text{N}$ values of the detritus of invasive seagrass in St Eustatius largely overlapped with conch, while there was less overlap with the seagrass detritus and conch in St Maarten (mixed native / invasive) and St Barthélemy (native), with conch having more enriched $\delta^{15}\text{N}$ values than seagrass detritus. The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of the detritus from the different seagrass species were mostly overlapping, although with some significant differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ among the different species. $\delta^{15}\text{N}$ values of *H. stipulacea* detritus were consistently more enriched compared to the detritus of native seagrass species, *i.e.* of *S. filiforme* detritus in St Eustatius ($p < 0.01$, $df = 1$) and St Maarten ($p < 0.05$, $df = 1$), as well as of *T. testudinum* in St Maarten ($p < 0.01$, $df = 1$) (Figure 5.2). *H. stipulacea* detritus also differed in $\delta^{13}\text{C}$ from the other species and showed more depleted $\delta^{13}\text{C}$ values compared to the detritus of *S. filiforme* in St Eustatius ($p < 0.01$, $df = 1$). In St Maarten the difference was reversed with $\delta^{13}\text{C}$ values of *H. stipulacea* detritus being more enriched compared to *S. filiforme* ($p < 0.01$, $df = 1$) and *T. testudinum* ($p < 0.01$, $df = 1$). *S. filiforme* detritus also had more enriched $\delta^{13}\text{C}$ values compared to *H. wrightii* ($p < 0.01$, $df = 1$) and *T. testudinum* ($p < 0.01$, $df = 1$) detritus in St Barthélemy.

Sediment

Organic matter from sediment samples was in general more depleted in $\delta^{13}\text{C}$ than the other food sources (Figure 5.2). All other food sources were between 1.77–8.77 ‰, 1.56–10.91 ‰, and 3.21–10.29 ‰ more enriched in $\delta^{13}\text{C}$ compared to organic matter from sediment from St Eustatius, St Barthélemy, and St Maarten, respectively (Figure 5.3).

Cyanobacteria

Three cyanobacteria mats (one red, likely *Blennothrix* spp., and two brown likely *Oscillatoria* spp.) found in St Eustatius were significantly ($p < 0.01$, $df = 2$) different from each other (Figure 5.2) in $\delta^{13}\text{C}$. With cyanobacteria red (CyR) being more depleted than cyanobacteria brown A (CyB A) ($p < 0.01$, $df = 1$) and B (CyB B) ($p < 0.01$, $df = 1$). CyB B was also more depleted than CyB A ($p < 0.01$, $df = 1$). For $\delta^{15}\text{N}$, CyR was significantly more depleted than CyB A ($p < 0.01$, $df = 1$) and CyB B ($p < 0.01$, $df = 1$) in $\delta^{15}\text{N}$.

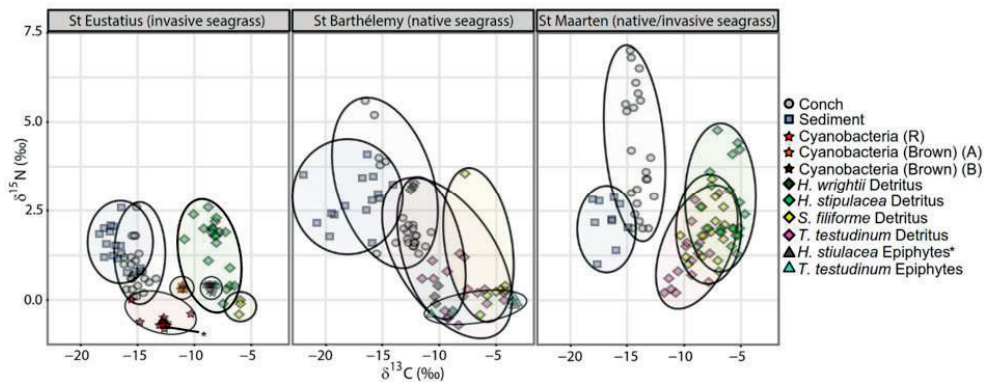


Figure 5.2. Iso-space plot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the conch and the potential food sources from the three sampling locations; St Eustatius (invasive seagrass), St Barthélemy (native seagrass) and St Maarten (mixed native / invasive seagrass). * = positioning of the single sample of *H. stipulacea* epiphytes and ellipses indicate the spread of the values of each item.

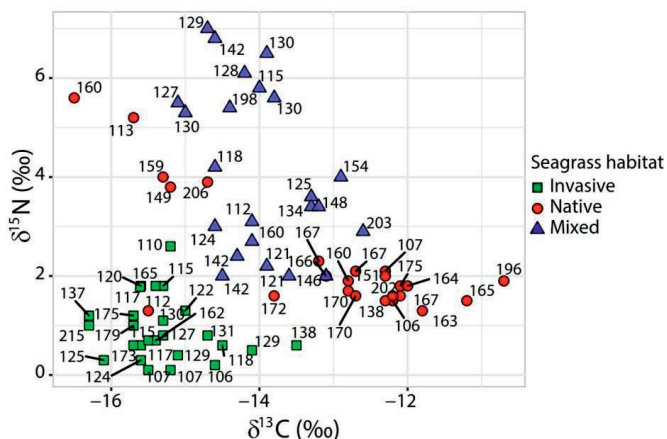


Figure 5.3. Juvenile queen conch (*Lobatus gigas*) values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from at the three seagrass habitats (invasive [St Eustatius], native [St Barthélemy], and mixed [St Maarten] with corresponding shell length (mm) indicated next to each point.

5.3.1.2 Diet composition

Conch had similar diets in all three habitats, with organic matter from the sediment being the primary dietary source, as apparent from the stable isotope mixing model (Figure 5.4). Median proportions of sediment organic matter ranged from 58.4% at St Barthélemy (95% credibility interval: 45.6–66.8%) to 68.5% (CI: 63.5–73.2%) at St Maarten, and 69.5% (CI: 61.9–77.7%) at St Eustatius (Figure 5.4). Seagrass detritus and seagrass epiphytes had a relatively small predicted contribution to the juvenile conch diet, irrespective of seagrass species (Figure 5.4). Cyanobacteria mats were only found in St Eustatius and had a median contribution to the juvenile queen conch diet of 3.6% (CI: 0.6–9.6%), 5.6% (CI: 0.7–16.2%), and 15.1% (CI: 2.7–28.0%) respectively (Figure 5.4).

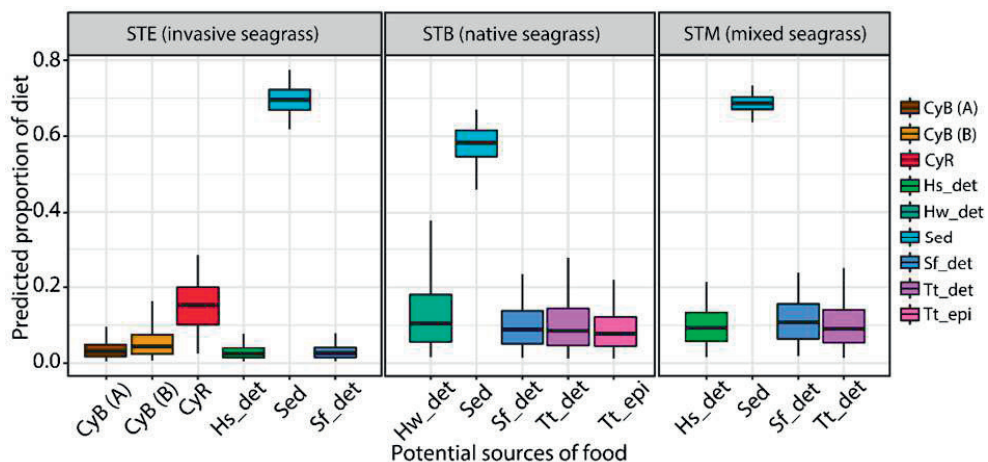


Figure 5.4. Median (horizontal line within the box), first and second quartile (boundaries of the box), and 95 % credibility interval (whiskers of the box) of predicted proportion of food source contribution to the diets of juvenile (shell length = 100 – 205 mm) queen conch (*Lobatus gigas*) at St Eustatius (majority invasive [$> 99\%$] seagrass), St Barthélemy (native seagrass), and St Maarten (mixed native / invasive seagrass) estimated by Stable Isotope Mixing Models in R ('simmr'). CyB (A) = Cyanobacteria (brown) species A, CyB (B) = Cyanobacteria (brown) species B, CyR = Cyanobacteria (red), Hs_det = *H. stipulacea* detritus (invasive seagrass), Hw_det = *H. wrightii* detritus (native seagrass), Sf_det = *S. filiforme* detritus (native seagrass), Tt_det = *T. testudinum* detritus (native seagrass), Tt_epi = *T. testudinum* epiphytes (native seagrass). Missing values indicated that the potential food source was not found in the particular location.

5.3.2 Growth

Nineteen juvenile conch in the native seagrass habitat showed positive growth rates ($> 0\text{ mm day}^{-1}$), with a maximum individual growth rate of 0.17 mm day^{-1} , while only 5 juvenile conch in the invasive seagrass habitat had positive growth rates, with a maximum of 0.03 mm day^{-1} (Figure 5.5). The predicted probability of positive growth rate in juvenile conch in the native seagrass bed was 0.63 (CI: 0.45–0.79), which was significantly higher than in the invasive seagrass bed, with a probability of 0.17 (CI: 0.07–0.35) (Figure 5.6).

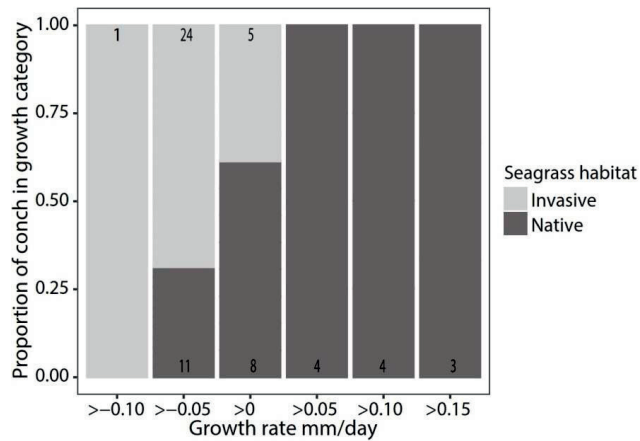


Figure 5.5. Proportion of juvenile conch of each habitat (native [dark grey] and invasive [light grey]) in each of the growth rate increment category. Numbers in the bottom (native) and top (invasive) shows the number (n) of conch in each growth increment category.

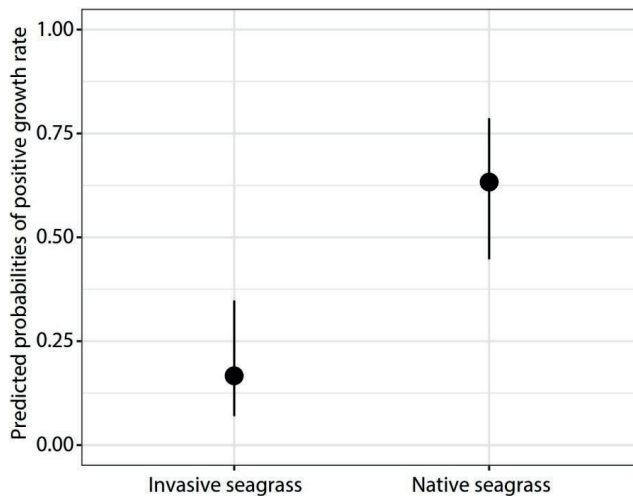


Figure 5.6. Predicted probabilities of positive growth rate in the invasive (*H. stipulacea*) and the native (*T. testudinum* and *S. filiforme*) seagrass with 95 % confidence interval.

5.4 Discussion

The similarities between habitats in the positioning of the stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of each potential food source and the juvenile conch (Figure 5.2), as well as the predicted dietary contributions of the mixing model, indicate comparable feeding patterns for juvenile conch in native, invasive and a mix of native and invasive seagrass, irrespective of sampling site. In all habitats the most important source of nutrition appears to be sediment organic matter (benthic diatoms and POM), despite differences between the habitats in variables such as depth, distance to shore, and anthropogenic nutrient input (Figure 5.2, 5.4).

The importance of the organic material in the sediment for juvenile queen conch was already suggested by Stoner and Waite (1991), who found large amounts of sediment in the stomach of juvenile conch. Similar findings have also been reported for *Laevistrombus canarium*, another Strombidae species, of which juveniles feed up to 40 % on surface sediment (Husna et al. 2017). Stoner & Waite (1991) further showed that macroalgae (particularly *Laurencia* spp. and *Batophora oerstedii*) were the most likely food source of juvenile conch in the shell length range of 120 – 140 mm, found in native seagrass (*T. testudinum*, and *S. filiforme*) beds in the Bahamas. However, the general importance of macroalgae to the diet of juvenile conch in our study area could not be confirmed as these macroalgae species were not found at any of the survey sites in this study. Several studies have also indicated that seagrass detritus is an important food source for juvenile queen conch, in particular detritus of *T. testudinum* (Stoner 1989b, Stoner & Waite 1991). While this study indicated that detritus is likely a food source for juvenile conch (Figure 5.2 ,5.4), the importance of this source in our study area appears to be less than previously attributed by Stoner & Waite (1991).

Although the organic material in the sediment was found to be the most important food source for juvenile conch, this study also shows that the organic material in the sediment was not the sole source of carbon and nitrogen for juvenile conch in any of the habitats. In addition, there were differences in food items between the sites, depending on their availability, confirming previous suggestions that juvenile conch are partly opportunistic in their feeding pattern (Robertson 1961, Randall 1964, Stoner & Waite 1991). Different food

items have also been found in the stomach of juvenile conch, giving further support to the suggestion of a mixed diet (Stoner & Waite 1991, Stoner & Sandt 1992). In St Eustatius (invasive site) a mix of sediment and a species of red cyanobacteria (likely *Blennothrix spp.* or *Oscillatoria spp.*) were identified as food source for juvenile conch. At this site three distinct cyanobacteria mats were found, but the two brown cyanobacteria mats seem to have contributed little to the diet of conch (Figure 5.4). This indicates a possible preference for conch for specific species of cyanobacteria as a source of nutrition. Both the detritus of native seagrass (*S. filiforme*) and invasive seagrass (*H. stipulacea*) were unlikely food sources for conch in St Eustatius, given the large separation between $\delta^{13}\text{C}$ of conch and the seagrass detritus ($>7.3\text{‰}$, $>2.4\text{‰}$, respectively) (Figure 5.2, 5.4). In contrast, for at least part of the conch in St Barthélemy (native site) and St Maarten (mixed site), a contribution to the diet from seagrass detritus was likely (Figure 5.2, 5.4). The proportional contribution of detritus to the diet of conch from the native and the invasive species remains uncertain, due to the overlap in the isotopic signatures of the detritus of the different seagrass species (Figure 5.2, 5.4). Even though there were significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between the detritus of the different seagrass species in St Eustatius, St Barthélemy, and St Maarten, this was not reflected in any distinct difference in the contribution of the seagrass species' detritus to the diet of juvenile conch (Figure 5.4). *H. stipulacea* has so far been found in depths from <1 to 30 m in the Caribbean (Willette et al. 2014, Scheibling et al. 2018) and was found in abundance at the sampling site in St Eustatius (invasive, 14–20 m depth) and St Maarten (mixed, 4–6 m depth) (Table 5.1) and its detritus was abundant at both sites (St Eustatius and St Maarten). However, it was unlikely to contribute to the diet of conch in St Eustatius and its contribution to the diet of conch in St Maarten appears to be limited (Figure 5.2, 5.4). This raises the question whether there is a difference in nutritional value of detritus from different species of seagrass for juvenile conch and if detritus of *H. stipulacea* can be utilized by conch as a source of nutrition to any extent.

In comparison to St Eustatius, more enriched $\delta^{15}\text{N}$ values were found in conch from the collection sites at St Barthélemy and St Maarten (Figure 5.1). Higher $\delta^{15}\text{N}$ values may be caused by the restricted water flow and by higher anthropogenic nutrient pollution (Tett et al. 2003), which is the case for both sampling sites at St Barthélemy and St Maarten. Both sites were close to outlets from enclosed water bodies as well as hotels with ca. 120 and 650

hotel rooms in St Barthélemy and St Maarten respectively. The site in St Eustatius was located further from shore (Table 5.1) and anthropogenic sources of nitrogen were considerably fewer (hotel rooms ca. 40) (Figure 5.1) (pers. obs). Therefore, the more enriched values of $\delta^{15}\text{N}$ of conch in St Maarten and in St Barthélemy are likely to be at least partly a consequence of a higher $\delta^{15}\text{N}$ baseline caused by more available anthropogenic nitrogen. This was particularly evident in St Maarten where all the conch with a $\delta^{15}\text{N}$ isotope signature $>5\text{‰}$ were collected close to a large source of anthropogenic nitrogen (outlet from Simpson Bay lagoon) (Lips & van Slooten 2009) (Figure 5.3). Previous studies have also found $\delta^{15}\text{N}$ in gastropods to reflect the exposure of sites to anthropogenic nitrogen, with a higher $\delta^{15}\text{N}$ baseline at impacted compared to pristine sites (Vermeulen et al. 2011).

Our growth experiment showed that there was a higher probability of positive growth rates in the native seagrass compared to the invasive seagrass (0.63 vs. 0.17). Even if conch in the invasive seagrass did grow, none had growth rates comparable to previously recorded high summer growth rates at nursery sites in native habitats in the Wider Caribbean Region (0.06–0.54 mm day⁻¹) (Randall 1964, Weil & Laughlin 1984, Iversen et al. 1987, Wicklund et al. 1988, Stoner 1989a,b, Stoner & Sandt 1992). In contrast, 11 of the 30 conch in the native seagrass did show such high growth rates, suggesting that the native seagrass habitat had a higher potential for higher growth of juvenile conch. The observed negative growth rates are not unique and have been linked to low food abundance, habitat quality, and shell erosion (Stoner 1989b, Stoner & Sandt 1991). Due to the relatively short duration of the experiment (47 days) the negative growth rates were unlikely to be due to shell erosion and more likely due to poor habitat quality and low food abundance (Stoner 1989b, Stoner & Sandt 1991). Even though the growth experiment had a shorter duration than desired, the results suggest higher growth rates of juvenile conch in native compared to invasive seagrass beds, at least under conditions in which other sources of non-seagrass related food items are not available (i.e. cyanobacteria, macroalgae, such as *Laurencia* spp. and *Batophora oerstedii*) (Stoner & Sandt, 1991).

A possible reason for the poorer growth performance of juvenile queen conch in invasive seagrass compared to native seagrass is reduced quality of, access to, and/or availability of food sources in invasive seagrass meadows. This could be related to the vegetation density

and growth strategy of *H. stipulacea*. In the Caribbean, *H. stipulacea* often creates dense continuous mats with little or no sediment exposed, especially under high nutrient conditions (van Tussenbroek et al. 2016) and with a dense maze of rhizomes often on top of the sediment surface. Native *T. testudinum* usually grows to seagrass shoot densities between 300–1200 shoots m⁻² (Linton & Fisher 2004), while shoot densities of *H. stipulacea* can often be between 3–5 times higher (ca. 3500 - 5500 shoots m⁻²) (Becking et al. 2014). Such dense *H. stipulacea* beds are found throughout the species' range in the Caribbean at depths ranging from 1 - 30 m (Willette et al. 2014, Scheibling et al 2018), and seagrass shoot densities of *H. stipulacea* were also high in the experimental enclosures in this study, while total seagrass shoot densities in the enclosures at the native seagrass bed were approximately 2 to 3 times lower (Table 5.2). In dense seagrass beds of *H. stipulacea* the area of exposed sediment will be lower compared to most beds of *T. testudinum* *S. filiforme*, and other native seagrass species. Due to the importance of the organic material in the sediment as a source of nutrition for juvenile conch, a restricted access to the sediment, when other sources of nutrition are not readily available (e.g. macroalgae, high epiphytic growth, *T. testudinum* detritus, cyanobacteria), could affect growth negatively as observed in this study. This is corroborated by Stoner and Sandt (1991), who showed that juvenile conch, transplanted to sites of high accumulation of detritus and native seagrass biomass, similar to the conditions of the invasive seagrass beds in this study, also showed lower growth compared to sites with low and medium native seagrass biomass. This suggests that high biomass seagrass beds, both native and invasive, can create unfavorable conditions to support high juvenile conch growth rates. Restricted benthic foraging driven by *H. stipulacea* and other habitat-forming invasive species (e.g. the macroalgae *Caulerpa taxifolia*) have also been observed in fish (e.g. *Mullus surmuletus*) in the Caribbean and elsewhere (Longepierre et al. 2005, Olinger et al. 2017). Furthermore, the dense canopy structure of *H. stipulacea* reduces the amount of light reaching the sediment surface which may negatively affect benthic diatom production (Hill 1996, Yang & Flower 2012), thereby reducing the abundance of potential food sources for queen conch in the sediment (i.e. benthic diatoms) (Stoner & Waite 1991). In addition, *H. stipulacea* appears to respond favorably to high nutrient conditions (Tussenbroek et al. 2016), whereas *T. testudinum* responds negatively (Tomasko et al. 1996). High nutrient conditions may thus exacerbate the possible negative effects of

high-density *H. stipulacea* to growth rates of juvenile queen conch, while simultaneously negatively affecting areal coverage of native seagrasses (i.e. *T. testudinum*).

Although we postulate that the dense growth pattern of *H. stipulacea* is the cause of the observed lower growth of juvenile conch in invasive seagrass, there were potential differences in environmental characteristics (i.e. nutrient level, sediment grain size) between the enclosure sites which could have influenced growth. Higher nutrient availability possibly occurred in the invasive seagrass compared to the native seagrass site, due to differences in distance to a large source of anthropogenic nitrogen (i.e. Simpson Bay lagoon). The invasive seagrass site was located closer to the outlet of Simpson Bay lagoon (350m) than the enclosures at the native seagrass site (850 m). Higher nutrient availability e.g. from anthropogenic sources may change the composition of the benthic diatom community (Licursi et al. 2016), which could have affected growth. However, the results from the stable isotope analysis showed a similar diet across all investigated seagrass habitats with sediment as the most important source. This was not influenced by differences in habitat characteristics, suggesting that conch can utilize various diatom communities as a source of nutrition, making differences in diatom community less likely to influence conch growth. Benthic diatom abundance, species composition and nutritional quality in relation to environmental parameters such as water quality and grain size were beyond the scope of this study and their possible effects on queen conch growth remains therefore uncertain.

Although *H. stipulacea* is more sensitive to strong currents and wave action than native seagrasses (den Hartog 1970, Steiner & Willette 2013, Scheibling et al. 2018) and therefore also more vulnerable in shallow waters, the expansion of *H. stipulacea* in the Wider Caribbean Region will continue and likely at the expense of native seagrass beds (Willette & Ambrose 2012), especially in deeper waters and sheltered areas. In areas where native seagrass species will be replaced by *H. stipulacea*, it is to be expected that the growth of juvenile queen conch will deteriorate, due to the growth pattern of *H. stipulacea*, which potentially limits access to and/or reduces the abundance of important food sources (i.e. benthic diatoms and POM). Furthermore, the stable isotope analysis and the mixing model showed that conch found in areas with only invasive seagrass (St Eustatius) did not derive nutrition from the detritus of *H. stipulacea* to any considerable extent, indicating that *H.*

stipulacea detritus cannot be considered a replacement of native food sources such as *T. testudinum* detritus.

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Supplementary material

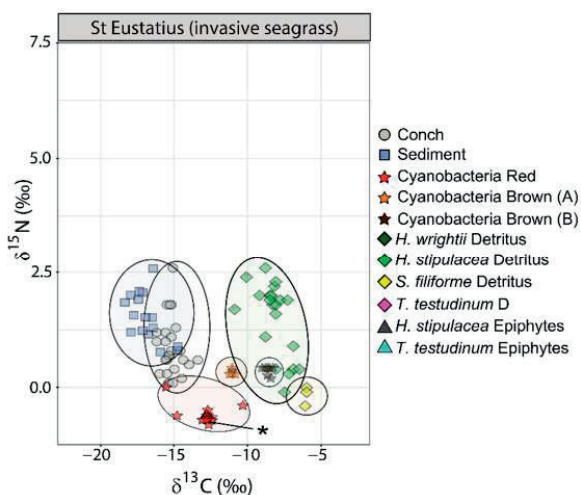


Figure S5.1. Iso-space plot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the conch and the potential food sources from St Eustatius (invasive seagrass). * = positioning of the single sample of *H. stipulacea* epiphytes and ellipses indicate the spread of the values of each item.

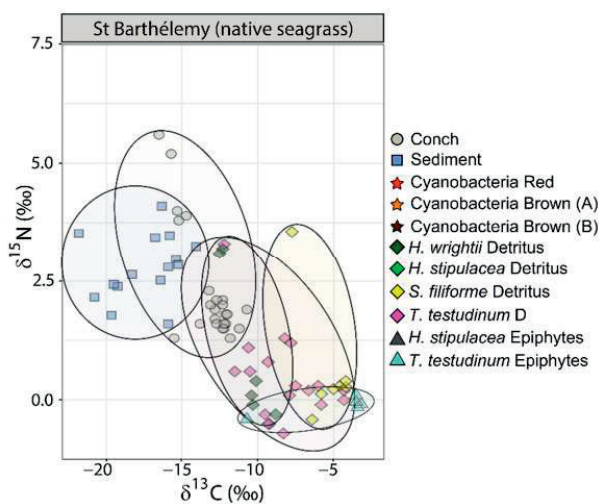


Figure S5.2. Iso-space plot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the conch and the potential food sources from St Barthélemy (native seagrass). Ellipses indicate the spread of the values of each item.

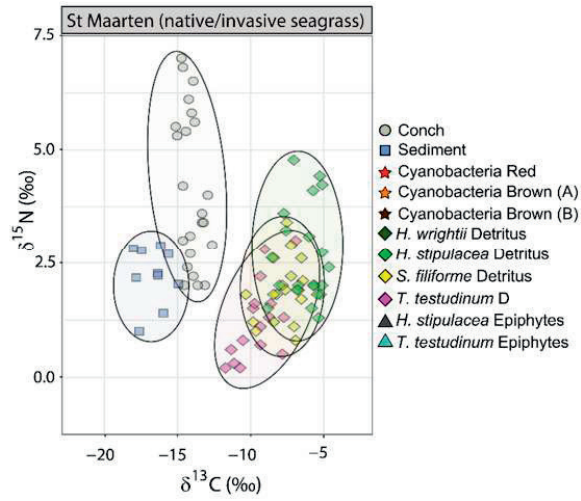


Figure S5.3. Iso-space plot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the conch and the potential food sources from St Maarten (invasive seagrass). Ellipses indicate the spread of the values of each item.

Chapter 6

Isolation by oceanic distance and spatial genetic structure in an overharvested international fishery

Truelove, N., Box, S.J., Aiken, C.J., Blythe-Mallett, A., Boman, E.M., Booker, C.J., Byfield, T.T., Cox, C.E., Davis, M.H., Delgado, G.A., Glazer, B.A., Griffiths, S.M., Kitson-Walters, K., Kough, A., Pérez Enríquez, R., Preziosi, R.F., Roy, M.E., Segura-García, Iris., Webber, M.K., Stoner, A.W. (2017)

Diversity and Distributions. 1–9

Abstract

Aim: A detailed understanding of spatial genetic structure (SGS) and the factors driving contemporary patterns of gene flow and genetic diversity are fundamental for developing conservation and management plans for marine fisheries. We performed a detailed study of SGS and genetic diversity throughout the overharvested queen conch (*Lobatus gigas*) fishery. Caribbean countries were presented as major populations to examine transboundary patterns of population differentiation.

Location: Nineteen locations in the greater Caribbean from Anguilla, the Bahamas, Belize, Caribbean Netherlands, Honduras, Jamaica, Mexico, Turks and Caicos, and the USA.

Methods: We genotyped 643 individuals with nine microsatellites. Population genetic and multivariate analyses characterized SGS. We tested the alternate hypotheses: (1) SGS is randomly distributed in space or (2) pairwise genetic structure among sites is correlated with oceanic distance (IBOD).

Results: Our study found that *L. gigas* does not form a single panmictic population in the greater Caribbean. Significant levels of genetic differentiation were identified between Caribbean countries ($F_{CT} = 0.011$; $p = 0.0001$), within Caribbean countries ($F_{SC} = 0.003$; $p = 0.001$), and among sites irrespective of geographic location ($F_{ST} = 0.013$; $p = 0.0001$). Gene flow across the greater Caribbean was constrained by oceanic distance ($p = 0.0009$; Mantel $r = 0.40$), which acted to isolate local populations.

Main conclusions: Gene flow over the spatial scale of the entire Caribbean basin is constrained by oceanic distance, which may impede the natural recovery of overfished *L. gigas* populations. Our results suggest a careful blend of local and international management will be required to ensure long- term sustainability for the species.

6.1 Introduction

A detailed understanding of spatial genetic structure (SGS) and the factors driving contemporary patterns of gene flow are fundamental for understanding marine species' responses to fishing pressure, habitat destruction, and climate change (Bay & Palumbi, 2014; D'Aloia, Bogdanowicz, Harrison, & Buston, 2014; D'Aloia et al., 2015; Pinsky & Palumbi, 2014). Likewise, knowledge of SGS is necessary for informing conservation approaches, such as identifying ecologically significant units (Palsbøll, Berube, & Allendorf, 2007), identifying the appropriate spatial scale of marine protected areas (Gaines, White, Carr, & Palumbi, 2010), and fostering international relations to conserve species whose ranges span geopolitical boundaries (Kough, Paris, & Butler, 2013; Truelove et al., 2015). Understanding the ecological and physical drivers of SGS is complicated due to the bipartite life histories of marine species (D'Aloia et al., 2014). For example, the adults of many benthic marine species are primarily sedentary with the majority of dispersal occurring during a pelagic larval phase that is subjected to prevailing ocean currents (Selkoe, Henzler, & Gaines, 2008). As the resilience of marine species to anthropogenic pressure is strongly linked to the degree of connectivity within metapopulations (Kritzer & Sale, 2004), a great deal of research has focused on developing genetic and biophysical models to quantify larval dispersal and thus understand how it shapes SGS (Selkoe, Gaggiotti, Bowen, & Toonen, 2014). For example, the biophysical modelling studies of the coastal boundary layer (CBL)—a prominent feature in the coastal ocean with reduced velocities due to friction with the shore suggest that self-retention of larvae is common in the CBL, regardless of the length of a species' pelagic larval duration (PLD; Nickols, White, Largier, & Gaylord, 2015). Therefore, nearshore processes such as those associated with the CBL may have a profound effect on SGS, particularly in benthic marine species that spawn in shallow nearshore habitats. *Lobatus gigas* is a large benthic marine gastropod (shell length can exceed 30 cm) of high economic and cultural importance that form spawning aggregations in shallow seagrass and sandplain habitats throughout the greater Caribbean (Mueller & Stoner, 2013; Randall, 1964). The species is heavily fished and is the basis of a lucrative export market to the United States and Europe (Acosta, 2006). *Lobatus gigas* is harvested in over 25 Caribbean nations and territories and populations have experienced significant declines throughout its range due to overfishing (Stoner, Davis, & Booker, 2012a). For example, the fishery collapsed in Florida and has yet to recover despite

nearly a 30- year ban on fishing and active restoration efforts (Delgado & Glazer, 2007). In addition, *L. gigas* was listed on Appendix II of the Convention on International Trade of Endangered Species of Flora and Fauna (CITES) in 1990 (Acosta, 2006). By 2004, the CITES Authority suspended trade in the Dominican Republic, Haiti, Honduras, Antigua and Barbuda, Barbados, Dominica, and Trinidad and Tobago based on evidence of declining stock or lack of an effective management framework (Acosta, 2006). As such, elucidating the SGS of *L. gigas* in the Caribbean is vital, especially if it can improve management practices (e.g., regional vs. local management efforts). Early genetic studies of *L. gigas* using electrophoretic methods found a high degree of gene flow among populations dispersed over the species' geographic distribution, with definitive separation observed only between populations in Bermuda and those in the Caribbean basin (Mitton, Berg, & Orr, 1989). At the local level, Perez- Enriquez, Garcia- Rodriguez, Mendoza- Carrion, and Padilla (2011) and Zamora- Bustillos, Rodríguez- Canul, García de León, and Tello-Cetina (2011) found with mitochondrial DNA and microsatellites, respectively, low genetic differentiation among locations in the Mexican Caribbean. In the Florida Keys and Bimini, Campton, Berg, Robinson, and Glazer (1992) also found low genetic differentiation. Although Mitton et al. (1989) found limited evidence of population structure in the Caribbean, the authors hypothesized that the complex ocean currents of the Caribbean might restrict gene flow among Caribbean populations, even though larvae may disperse long distances throughout the Caribbean during their 16–28 days PLD (Davis, Bolton, & Stoner, 1993). Over the last decade, advances in biophysical modelling and seascape genetics suggest that larval behavior of marine species, coupled with the complex hydrodynamics of the marine environment, may limit gene flow leading to fine-scale patterns of SGS (D'Aloia et al., 2015; Iacchei et al., 2013; Selkoe et al., 2010; Thomas et al., 2015). These findings suggest that a more detailed examination of *L. gigas* population connectivity is warranted. In this study, we used microsatellite markers and a comprehensive sampling strategy to perform a detailed study of SGS of *L. gigas* across the greater Caribbean seascape. First, we conducted basic population genetic analyses to determine if there is evidence for population differentiation among localities within and between Caribbean countries. Second, we used multivariate analysis to visualize SGS. Third, we tested the alternative hypotheses: (1) SGS is randomly distributed in space or (2) pairwise genetic structure among sites is correlated with oceanic distance (IBOD).

6.2 Methods

6.2.1 Sample collection

We characterized SGS of *L. gigas* across the greater Caribbean sea-scape using nine microsatellites and sampling 643 individuals from 19 locations (Figure 6.1). Small pieces of mantle tissue ($< 1 \text{ cm}^2$) were excised and preserved in 95% ethanol or placed on filter paper for drying. Genomic DNA was extracted using the Qiagen DNeasy kit. Samples for Alacranes reef were processed as described in Perez- Enriquez et al. (2011).

6.2.2 Microsatellite genotyping

Queen conch was genotyped using nine polymorphic microsatellite loci (Truelove, Fai Ho, Preziosi, & Box, 2016; Zamora- Bustillos, Rodríguez- Canul, & De León, 2007). Genotyping was performed using an ABI 3730xl automatic DNA sequencer (Applied Biosystems) at the Smithsonian Institute's Laboratory of Analytical Biology. Microsatellite alleles were scored manually with GENEMAPPER® v3.7 software package (Applied Biosystems).

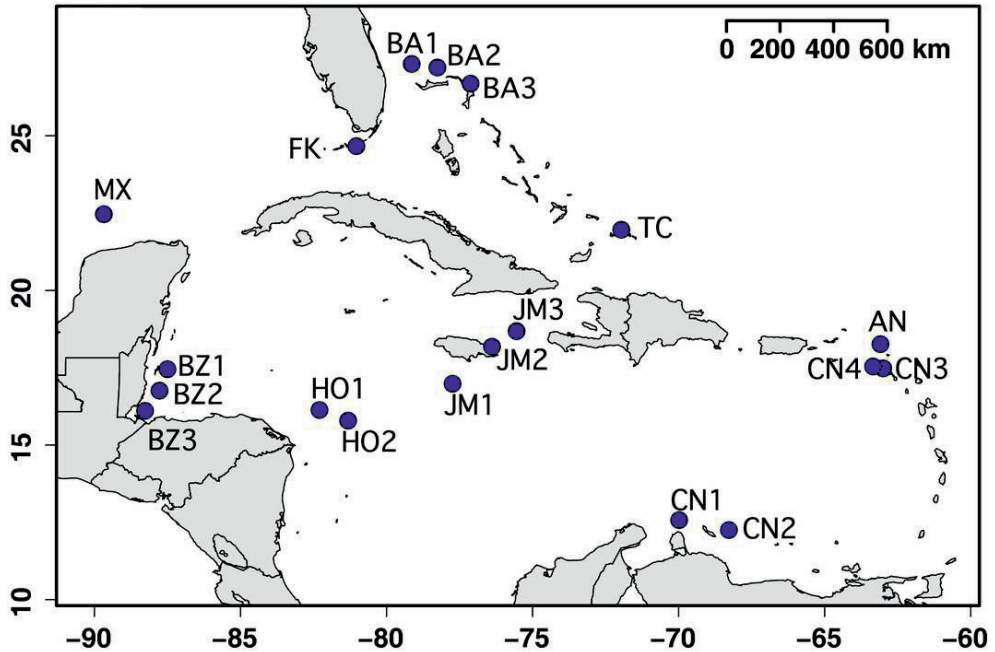


Figure 6.1. Map of the greater Caribbean showing locations of the *Lobatus gigas* sampling sites in blue. Site codes are FK, Delta Shoal, Florida; MX, Alacranes Reef, Mexico; BZ1, Lighthouse Atoll, Belize; BZ2, Glover's Reef, Belize; BZ3, Sapodilla Cayes, Belize; HO1, Banco Gordo, Honduras; HO2, Banco Oneida; JM1, Pedro Bank, Jamaica; JM2, Alligator Head, Jamaica; JM3, Formigas Bank; CN1, Caribbean Netherlands, Aruba; CN2, Caribbean Netherlands, Bonaire; CN3, Caribbean Netherlands, Saint Eustatius; CN4, Caribbean Netherlands, Saba; AN, Anguilla; TC, Turks and Caicos; BA1, Matanilla Shoal, Bahamas; BA2, Double Breasted Cay, Bahamas; and BA3, Whale Cay, Bahamas.

6.2.3 Data quality checks

Microsatellite alleles were binned with the R- package MSATALLELE version 1.02 (Alberto, 2009). Microsatellite loci were examined with MICROCHECKER 2.2.3 to check for patterns caused by null alleles, allele scoring error due to either large allele dropout or stutter, or other natural processes (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004). FREENA was used to assess bias in calculations of F_{ST} caused by null alleles (Chapuis & Estoup, 2007). Linkage disequilibrium was tested using GENEPOP with the values for the dememorization number, number of batches, and number of iterations per batch all set to 10K. (Raymond & Rousset, 1995; Rousset, 2008).

6.2.4 Genetic differentiation

A hierarchical AMOVA was run in GENODIVE (Meirmans, 2012; Meirmans & Van Tienderen, 2004) to identify differences between Caribbean nations/territories (FCT), among sites within Caribbean nations/territories (FSC), and among sites irrespective of political boundaries (FST). An infinite allele model was used based on Weir and Cockerham's (1984) calculations of FST (Weir & Cockerham, 1984), and the level of significance was tested using 50,000 permutations. Hedrick's G'ST was calculated in GENODIVE using 50,000 permutations. Hedrick's G'ST can be a more appropriate measure of genetic differentiation when heterozygosity is high, as is the case here, as it corrects for the tendency of FST to decline as polymorphism increases (Hedrick, 2005). The p-values for pairwise comparisons of genetic differentiation were calculated in GENODIVE with the log-likelihood G- statistic using 50,000 permutations. The false discovery rate multitest correction was used as a correction against Type I errors for all statistical analyses that included multiple comparisons (Benjamini & Yekutieli, 2001).

6.3 Results

6.3.1 Microsatellite analysis

There were 23 significant departures from Hardy–Weinberg Equilibrium HWE out of 171 comparisons across microsatellite loci and populations (Table S6.1). However, significant deviations from HWE were not consistently observed across loci or populations. All microsatellite loci were polymorphic, with effective number of alleles per population ranging from 5.24 to 7.39 (Tables S6.2 and S6.3). No evidence of significant linkage disequilibrium was observed among microsatellite loci. Analyses in MI- CROCHECKER suggested that deviations from HWE were not due to scoring error or large allele dropout. Although MICROCHECKER did not rule out the possibility of null alleles attributing to deviations from HWE; analysis with FREENA indicated that potential bias on calculations of global FST caused by null alleles was negligible (Global FST = 0.0141; Global FST with correction for null alleles = 0.0142). Therefore, all microsatellite loci were included in analyses of population structure.

Table 6.1. Pairwise matrix of above and pairwise F_{5T} below the diagonal. Significant values are displayed in bold after false discovery rate correction ($p < .0074$)

	BA1	BA2	BA3	TC	FK	MX	BZ1	BZ2	BZ3	HO1	HO2	JM1	JM2	JM3	CN1	CN2	CN3	CN4	AN
BA1																			
BA2	-0.007																		
BA3	0.005	0.002																	
TC	-0.001	0.000	0.009																
FK	0.009	0.006	0.015	0.007															
MX	0.050	0.040	0.021	0.047	0.050														
BZ1	0.013	0.012	0.012	0.008	0.019	0.037													
BZ2	0.010	0.011	0.018	0.009	0.019	0.048	0.003												
BZ3	0.010	0.008	0.015	0.004	0.013	0.039	-0.002	0.001											
HO1	-0.004	-0.001	0.013	-0.001	0.010	0.048	0.006	0.005	0.001										
HO2	-0.005	0.003	0.012	0.003	0.018	0.054	0.009	0.002	0.004	-0.003									
JM1	0.001	0.007	0.015	0.004	0.012	0.058	0.012	0.006	0.011	0.001	-0.002								
JM2	-0.004	0.000	0.012	0.005	0.020	0.047	0.012	0.011	0.007	-0.002	-0.003	0.009							
JM3	-0.001	0.001	0.010	0.003	0.013	0.050	0.010	0.007	0.009	0.001	0.002	0.005	0.001						
CN1	-0.002	0.005	0.013	0.006	0.014	0.041	0.014	0.013	0.011	0.003	0.000	-0.003	0.006	0.004					
CN2	0.009	0.020	0.031	0.020	0.027	0.073	0.028	0.014	0.030	0.018	0.007	-0.002	0.021	0.015	0.009				
CN3	-0.002	0.012	0.027	0.015	0.026	0.067	0.018	0.009	0.018	0.010	0.006	0.004	0.014	0.012	0.008	0.005			
CN4	0.000	0.004	0.021	0.009	0.019	0.065	0.025	0.012	0.022	0.011	0.004	0.003	0.013	0.005	0.005	0.005	0.006		
CN5	0.010	0.023	0.032	0.021	0.044	0.075	0.033	0.025	0.032	0.023	0.009	0.020	0.018	0.019	0.022	0.020	0.018	0.018	

6.3.2 Isolation-by-oceanic-distance

Euclidean distances may not accurately represent connectivity in the ocean, where islands and currents can impede routes of travel. We developed a simple measure of oceanographic distance between pairs of locations based on current fields from an operational ocean circulation model, Global HYCOM + NCODA assimilated (Chassignet et al., 2007). We calculated the average surface current velocity north and east from 2004 through 2013 during peak conch spawning (June and July; Aldana Aranda et al., 2014) within a rectangle bounded by the latitudes and longitudes of any pair of sites. The average current velocity north and east was then multiplied by a lower estimate of the duration of a conch's planktonic larval phase (14 days; Stoner, 2003) to come up with an oceanographic cost of traveling in each direction. These values were then added to the distance north and distance east, which were calculated using the Pythagorean theorem and the Euclidean distance as the hypotenuse. The Pythagorean theorem was applied again to calculate an oceanographic distance incorporating average decadal circulation through the area separating two points. Thus, a distance was increased if it was against the average current flow and reduced if it went with the current. We tested for correlations of genetic distance (F_{ST} and G'_{ST}) with oceanic distance, referred to as isolation- by- oceanic- distance (IBOD), in the R- package ADEGENET (Jombart, 2008) using a Mantel test with 10,000 permutations. A separate spatially explicit analysis of genetic variation was conducted using the spatial principal component analysis in the R-package ADEGENET (Jombart, 2008) following the methodology of Truelove et al. (2015). Briefly, we selected the first principal component that contained the highest levels of both spatial autocorrelation and genetic variance. Spatial patterns of genetic variation were visualized by color- coding the lagged score of the first principal component using the R ColorBrewer package (<http://colorbrewer2.org>).

6.3.3 Population structure

Microsatellite genetics identified significant levels of genetic differentiation between Caribbean countries ($F_{CT} = 0.011$; $p = 0.0001$), within Caribbean countries ($F_{SC} = 0.003$; $p = 0.001$), and among sites irrespective of its geographic location ($F_{ST} = 0.013$; $p = 0.0001$). Pairwise comparisons of F_{ST} ranged from -0.028 to 0.282 with 84 of 171 pairwise comparisons significant after FDR correction ($p < 0.0074$; Table 6.1). Comparisons of the two metrics of

population structure, F_{ST} and G'_{ST} , respectively, were highly correlated ($p < 2.2 \times 10^{-16}$; $R^2 = 0.99$). Principle coordinate analysis of site-specific levels of pairwise F_{ST} and G'_{ST} were both in agreement indicating that Alacranes Reef (Mexico), Florida Keys (USA), and Saint Eustatius (Caribbean Netherlands) were the most genetically divergent sites (Figure 6.2). The spatial principal component analysis using nine microsatellite loci suggested that queen conch in the eastern Caribbean was most differentiated from queen conch from sites in the western Caribbean in terms of positive spatial autocorrelation and genetic variance (Figure 6.3). Isolation- by- oceanic- distance analysis (Figure 6.4, Table S6.4) indicated that gene flow across the greater Caribbean was constrained by oceanic distance ($p = .0009$; Mantel $r = .40$). For simplicity, we reported F_{ST} and not G'_{ST} as the two metrics of population differentiation were highly correlated.

6.4 Discussion

This study advances our understanding of SGS in *L. gigas* throughout the greater Caribbean. The central finding of our study is that gene flow over the spatial scale of the entire Caribbean basin is constrained by oceanic distance. We identified significant genetic IBOD and found evidence of several regionally isolated populations throughout the greater Caribbean. The significant levels of IBOD provide indirect genetic evidence that the dispersal of *L. gigas* larvae is limited in multiple regions throughout the range of the species. *Lobatus gigas* does not form a single panmictic population in the Caribbean. Microsatellite genetics identified significant levels of genetic differentiation among Caribbean subregions (e.g., Florida Keys, Mesoamerican Barrier Reef, Lesser Antilles, Honduran/Jamaican Banks, Greater Antilles, and the Bahamas) and between the eastern and western Caribbean regions. These findings were supported by the spatial principal component analysis (Jombart, Devillard, Dufour, & Pontier, 2008). Site-specific pairwise comparisons of genetic differentiation found that the Florida Keys (USA), Saint Eustatius (Caribbean Netherlands), and Alacranes Reef (Mexico) were significantly differentiated from nearly all other sites in the Caribbean. These findings provide additional support for hypotheses generated by previous genetic studies of queen conch. Perez- Enriquez et al. (2011) hypothesized that clinal patterns in haplotype frequencies were caused by isolation- by- distance. Likewise, Mitton et al. (1989) hypothesized that Caribbean ocean

currents were largely responsible for the lack of panmixia observed in their study. Overall, the findings of our study provide genetic evidence of significant SGS that was explained by IBOD. Isolation- by- oceanic- distance was a key driver of the limited gene flow we observed among *L. gigas* populations. Likewise, Zhan et al. (2009) found ocean currents to be responsible for genetic differentiation in the Zhikong Scallop (*Clamys farreri*). These findings are corroborated by White et al. (2010), which used an IBOD approach to explain 50% of the genetic variance in the commercially harvested gastropod mollusc *Kelletia kelletii*. Teske, Sandoval- Castillo, van Sebille, Waters, and Beheregaray (2016) used the IBOD approach to reveal high levels of self-recruitment in the limpet *Siphonaria diemenensis* that was driven by low-velocity nearshore currents that acted to retain larvae locally.

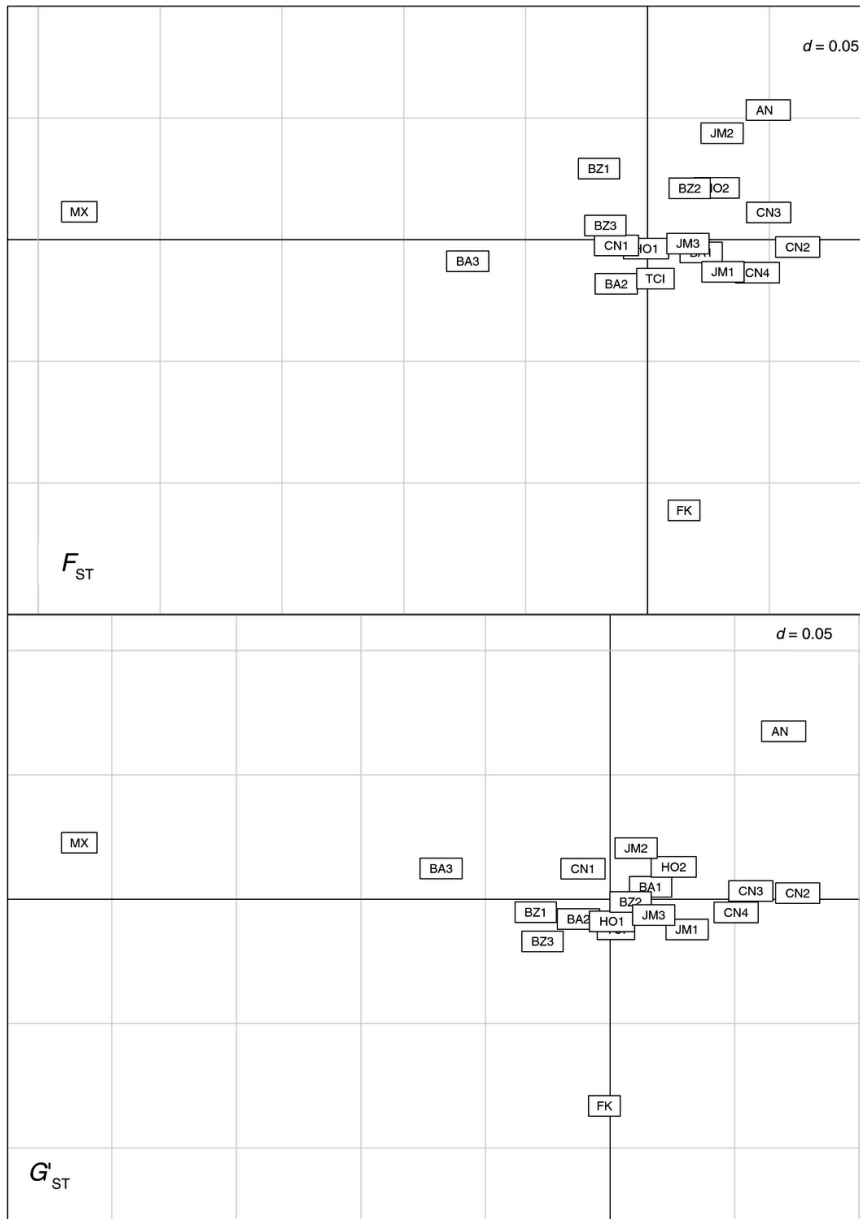


Figure 6.2. Principle coordinates analysis of site-specific pairwise comparisons of genetic differentiation, F_{ST} (top panel) and G'_{ST} (bottom panel). Site codes are FK, Delta Shoal, Florida; MX, Alacranes Reef, Mexico; BZ1, Lighthouse Atoll, Belize; BZ2, Glover's Reef, Belize; BZ3, Sapodilla Cayes, Belize; HO1, Banco Gordo, Honduras; HO2, Banco Oneida; JM1, Pedro Bank, Jamaica; JM2, Alligator Head, Jamaica; JM3, Formigas Bank; CN1, Caribbean Netherlands, Aruba; CN2, Caribbean Netherlands, Bonaire; CN3, Caribbean Netherlands, Saint Eustatius; CN4, Caribbean Netherlands, Saba; AN, Anguilla;

TC, Turks and Caicos; BA1, Matanilla Shoal, Bahamas; BA2, Double Breasted Cay, Bahamas; and BA3, Whale Cay, Bahamas.

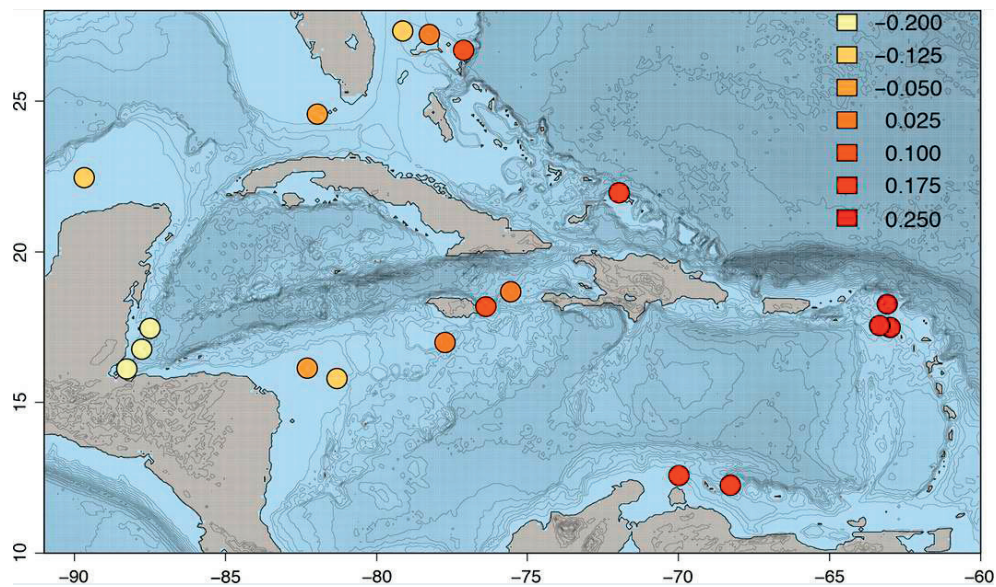


Figure 6.3. Map of large-scale spatial genetic structure of *Lobatus gigas* in the greater Caribbean. Principle component analysis of spatial genetic structure detected by ADEGENET with colors corresponding to the lagged score of the first principal component.

Biophysical modeling studies of marine species in the Caribbean with a similar PLD to *L. gigas* (e.g., corals and reef fish) suggest that larvae are likely to disperse among localities via the prevailing Caribbean current, which is largely continuous and unidirectional (Galindo, Olson, & Palumbi, 2006; Kool, Paris, Andréfouët, & Cowen, 2010; Purcell, Cowen, Hughes, & Williams, 2009). Persistent gyres and the CBL constitute important oceanographic mechanisms that promote local retention of larvae (Kough et al., 2013; Nickols et al., 2015). The significant levels of IBOD among *L. gigas* populations provides additional support to the growing body of evidence that oceanographic currents help explain SGS in high- gene flow marine species. The offshore banks of Honduras and Jamaica may play an important role in facilitating connectivity among Caribbean queen conch populations. Pairwise comparison of genetic differentiation and spatial principal component analysis provided evidence of long-distance gene flow (i.e., >

1,000 km) between *L. gigas* populations from Pedro Bank (Jamaica) and all sites in the Caribbean Netherlands. Likewise, long- distance gene flow was found among queen conch populations from the Honduran Banks, Bahamas, and Turks and Caicos. These findings suggest that the offshore banks of Honduras and Jamaica may be important stepping stones for facilitating gene flow among queen conch populations on the Caribbean wide scale.

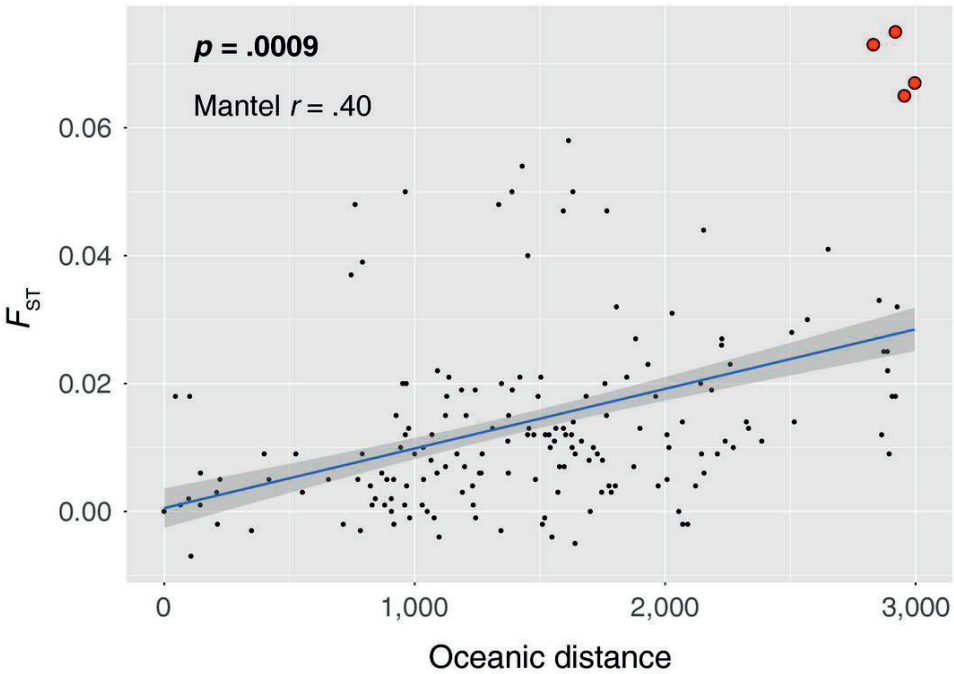


Figure 6.4. Scatter plot showing positive isolation-by-oceanic-distance between pairwise genetic differentiation (linearized F_{ST}) and oceanic distance (km) among *Lobatus gigas* sampling sites. Confidence intervals are visualized as the shaded areas above and below the blue trend line. The red dots indicate pairwise comparisons between Mexico and Anguilla, St. Eustatius, Saba, and Bonaire (starting at the red dot with the highest F_{ST} and moving clockwise).

Additional evidence of the importance of these offshore banks in facilitating long- distance connectivity comes from genetic research of spiny lobster fishery, which found high levels of gene flow between the Honduran Banks and lobster populations in Panama, Mexico, and Belize (Truelove et al., 2014). The offshore network of banks in Honduras, Nicaragua, and Jamaica remain poorly studied (Chollett, Stoyale, & Box, 2013), and more research is required to understand the role that this remote region of the Caribbean plays in the connectivity of

marine species. Biophysical modeling studies have shown coastal boundary features act to retain the bulk of larvae locally while a small, but significant, proportion can be exported vast distances (Butler, Paris, Goldstein, Matsuda, & Cowen, 2011; Nickols et al., 2015). The long-distant migrants at the tail of the dispersal kernel may provide sufficient levels of gene flow to mask SGS when using a small number of neutral nuclear genetic markers (Latch, Dharmarajan, Glaubitz, & Rhodes, 2006). This may explain the lack of significant pairwise comparisons of F_{ST} and G'_{ST} between Honduras and Turks and Caicos. Our findings of limited larval dispersal resulting from indirect genetic methods should be interpreted with caution (sensu Christie, Johnson, Stallings, & Hixon, 2010). The indirect genetic methods we use to assess the connectivity of *L. gigas*, such as F_{ST} based analyses, rely upon theoretical assumptions (e.g., Hardy–Weinberg equilibrium and drift–mutation equilibrium) that are often limited by a lack of statistical power for detecting ecologically relevant patterns of connectivity when faced with moderate to high levels of gene flow (Hellberg, 2009). Multivariate analyses such as the spatial principal component analysis should be more robust to these limitations as it requires no theoretical genetic assumptions (Jombart, 2008). While our study found significant levels of population differentiation and IBOD, there may have been ecologically relevant levels of SGS that went undetected. Our findings have direct implications for informing local and regional management practices for *L. gigas*. The limited dispersal potential of *L. gigas* larvae, as suggested by significant IBOD, may impede the natural recovery of overfished *L. gigas* populations. For example, this could be a key factor for explaining why the Florida Keys fishery has yet to rebound after 30 years of closure to fishing (Delgado et al., 2008). Previous research has posited that there are hydrodynamic processes that act as retentive mechanisms for locally produced queen conch larvae in the Florida Keys and that few larvae are coming from upstream sources (Delgado et al., 2008). Further evidence to support our hypothesis of IBOD- limited recovery comes from population declines in the Exuma Cays Marine Protected Area (MPA) in the Bahamas, despite decades of protection and high levels of MPA compliance (Stoner et al., 2012a). It has been suggested that queen conch conservation efforts focus on local management (Delgado et al., 2008; Paris, Aldana- Aranda, Pérez- Pérez, & Kool, 2008), and our results corroborate this assertion to a certain degree. We recommend that management of *L. gigas* be tailored toward localized stock structure in regions with the highest levels of genetic divergence such as in the Caribbean Netherlands,

Mexico, and Florida. In contrast, the remote offshore network of banks in the Nicaraguan rise—which includes the territorial seas of Honduras Nicaragua, Colombia, and Jamaica—is likely to be an important region for maintaining population connectivity over larger spatial scales that span international boundaries. Analyses of genetic diversity among sites identified a high variation in the effective number of alleles among sites. Anguilla and Mexico had the lowest effective number of alleles. These two sites had the highest levels of IBOD suggesting that oceanic isolation may have reduced levels of genetic diversity at these sites. Overall, the results of our study suggest that a careful blend of local and international management will be required to ensure long- term sustainability for the species throughout its range in the Caribbean. Future studies will be required to more accurately delineate stock boundaries, ESUs, and thoroughly investigate mechanisms responsible for the high variation in genetic diversity observed among *L. gigas* populations. For example, Pinsky and Palumbi (2014) found that overexploitation in highly abundant marine fishes lowered allelic richness on average of 12% compared to closely related species that are not overharvested. Future genetic research should apply this approach to understand how overharvest may impact the evolutionary potential of *L. gigas*. In addition, we expect that the next generation of higher resolution genomic techniques will be capable of identifying queen conch subpopulations harboring unique genes adapted to local environmental conditions (sensu Hemmer- Hansen et al., 2013) as well as improving our understanding of genetic changes caused by fishery-induced evolution. For example, fishers selectively harvesting the largest queen conch individuals have been hypothesized to be responsible for a smaller morphotype of *L. gigas* in the Bahamas, known locally a samba conch (Stoner, Davis, & Booker, 2012b). To conclude, our finding of significant isolation- by- oceanic- distance suggests that queen conch fisheries cannot rely solely on outside sources of larvae to rebuilt overfished stocks.

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Supplementary material

Table S6.1: Departures from Hardy Weinberg Equilibrium (HWE). The table below shows the *P*-values for each combination of sampling location and microsatellite locus. Significant departures from HWE, after the sequential goodness-of-fit correction for multiple tests are shown in bold ($P < 0.008$).

	Sgig1	Sgig2	Sgig6	Conch17	Conch21	Conch23	Conch29	ConchPR1	ConchPR11
Mantanilla Shoal	0.012	0.231	0.277	0.387	0.019	0.017	0.198	0.008	0.203
Double Breaster's Cay	0.000	0.290	0.014	0.048	0.525	0.000	0.001	0.000	0.281
Whale Cay	0.019	0.388	0.007	0.088	0.271	0.000	0.216	0.432	0.410
Turks and Caicos	0.000	0.243	0.461	0.212	0.297	0.084	0.524	0.013	0.262
Florida Keys, Delta Shoal	0.106	0.402	0.588	0.562	0.594	0.183	0.013	0.000	0.056
Alacranes Reef	0.001	0.500	0.098	0.587	0.264	0.374	0.042	0.212	0.000
Lighthouse Atoll	0.507	0.028	0.580	0.063	0.080	0.371	0.205	0.217	0.204
Glover's Reef Atoll	0.254	0.001	0.022	0.097	0.080	0.016	0.220	0.198	0.172
Sapodilla Cayes	0.014	0.002	0.042	0.024	0.072	0.183	0.310	0.548	0.188
Banco Gordo	0.042	0.235	0.019	0.243	0.409	0.401	0.004	0.136	0.032
Banco Oneida	0.103	0.127	0.223	0.364	0.411	0.000	0.001	0.208	0.110
Pedro Bank	0.010	0.507	0.362	0.381	0.048	0.015	0.556	0.022	0.000
Alligator Head	0.012	0.322	0.216	0.168	0.601	0.000	0.144	0.000	0.288
Formigas Bank	0.001	0.307	0.024	0.185	0.519	0.019	0.105	0.000	0.010
Aruba	0.001	0.116	0.283	0.141	0.628	0.003	0.360	0.043	0.078
Bonaire	0.045	0.270	0.543	0.037	0.504	0.266	0.359	0.016	0.023
Saint Eustatius	0.217	0.397	0.270	0.327	0.514	0.073	0.014	0.002	0.196
Saba	0.570	0.058	0.424	0.498	0.449	0.118	0.015	0.010	0.478
Anguilla	0.176	0.074	0.339	0.483	0.475	0.034	0.466	0.118	0.152

Table S6.2. Summary statistics including the country, sampling site name, number of samples (N_s), effective number of alleles (EN_A), observed heterozygosity (H_o), total expected heterozygosity (H_T), and inbreeding coefficient (G_{IS}).

Country	Site Name	N_s	EN_A	H_o	H_T	G_{IS}
Bahamas	Mantanilla Shoal	26	6.374	0.656	0.757	0.133
Bahamas	Double Breaster's Cay	53	6.822	0.667	0.788	0.154
Bahamas	Whale Cay	26	6.709	0.707	0.807	0.124
Turks and Caicos	Turks and Caicos	47	6.905	0.726	0.778	0.067
USA	Florida Keys, Delta Shoal	23	6.202	0.714	0.783	0.088
Mexico	Alacranes Reef	34	5.290	0.679	0.775	0.124
Belize	Lighthouse Atoll	30	7.270	0.861	0.818	-0.053
Belize	Glover's Reef Atoll	26	6.565	0.822	0.778	-0.057
Belize	Sapodilla Cayes	29	6.921	0.904	0.802	-0.127
Honduras	Banco Gordo	38	6.575	0.684	0.755	0.094
Honduras	Banco Oneida	28	6.200	0.656	0.749	0.124
Jamaica	Pedro Bank	27	6.160	0.629	0.734	0.143
Jamaica	Alligator Head	21	6.614	0.632	0.790	0.200
Jamaica	Formigas Bank	88	7.387	0.730	0.786	0.070
Caribbean Netherlands	Aruba	20	5.530	0.670	0.742	0.098
Caribbean Netherlands	Bonaire	36	6.291	0.643	0.707	0.090
Caribbean Netherlands	Saint Eustatius	32	6.585	0.688	0.718	0.042
Caribbean Netherlands	Saba	32	6.370	0.680	0.725	0.063
Caribbean Netherlands	Anguilla	27	5.236	0.722	0.687	-0.052

Table S6.3. Microsatellite summary statistics including locus, (EN_A), observed heterozygosity (H_o), total expected heterozygosity (H_T), and inbreeding coefficient (G_{IS}).

Locus	N_A	EN_A	H_o	H_T	G_{IS}
Sgig1	19	4.237	0.632	0.790	0.191
Sgig2	19	2.919	0.699	0.681	-0.047
Sgig6	15	2.415	0.569	0.606	0.046
Conch17	25	5.739	0.852	0.837	-0.015
Conch21	19	6.026	0.843	0.850	0.007
Conch23	25	1.549	0.251	0.412	0.301
Conch29	26	7.392	0.811	0.884	0.079
ConchPR1	37	12.413	0.831	0.945	0.113
ConchPR11	35	13.201	0.896	0.947	0.049

Table S6.4. Pairwise comparisons of derived oceanic distance among sites calculated with a larval duration of 14 days calculated using the Global HYCOM + NCODA oceanographic models.

	BA1	BA2	BA3	TC	FK	MX	BZ1	BZ2	BZ3	HO1	HO2	JM1	JM2	JM3	CN1	CN2	CN3	CN4	AN
BA1		71	202	922	592	1036	1263	1362	1462	1034	970	1086	1018	987	1769	1893	1974	1939	1928
BA2	106		157	840	455	1125	1327	1425	1526	1096	1056	1079	997	950	1714	1832	1887	1852	1840
BA3	222	97		706	452	1247	1392	1491	1593	1146	1117	1042	936	867	1606	1717	1748	1714	1701
TC	980	907	790		1031	1901	1768	1851	1939	1336	1259	849	615	515	922	983	942	907	911
FK	1269	1089	926	1123		709	814	925	1036	844	830	847	889	914	1657	1816	2096	2061	2072
MX	1388	1451	1504	1767	962		490	617	720	785	900	1214	1390	1479	2157	2344	2770	2735	2773
BZ1	1564	1603	1626	1696	1187	746		140	207	286	380	663	870	1043	1631	1829	2296	2257	2333
BZ2	1629	1666	1684	1729	1241	762	209		184	416	502	800	932	1078	1641	1839	2388	2345	2385
BZ3	1713	1750	1766	1801	1310	791	212	144		410	534	923	1044	1172	1663	1861	2494	2452	2480
HO1	1548	1519	1456	1243	1034	1335	868	773	880		165	417	551	660	1005	1205	1873	1833	1881
HO2	1640	1572	1476	1190	1128	1429	1000	906	967	348		442	537	628	862	1067	1794	1756	1805
JM1	1234	1199	1123	823	1069	1614	1451	1374	1372	830	715		218	217	607	800	1368	1324	1377
JM2	1097	1050	962	655	966	1593	1536	1558	1578	917	783	399		222	617	787	1217	1181	1320
JM3	1078	1030	944	552	976	1632	1541	1597	1641	960	842	418	64		609	765	1159	1123	1247
CN1	2070	2008	1899	1259	2069	2650	2324	2333	2386	1785	1701	1344	1265	1231		203	859	840	922
CN2	2209	2142	2028	1346	2227	2831	2506	2516	2568	1962	1875	1510	1420	1375	525		794	783	867
CN3	2090	2007	1882	1205	2225	2997	2906	2894	2919	2272	2155	1776	1633	1522	1065	916		185	214
CN4	2054	1972	1847	1168	2185	2955	2872	2864	2888	2240	2121	1747	1594	1482	1035	889	144		179
CN5	2015	1931	1805	1136	2153	2919	2854	2887	2926	2260	2145	1759	1493	1389	1091	952	44	101	

Chapter 7

Biology, monitoring, and management of a tropical marine gastropod: the Queen conch (*Lobatus gigas*) in the Caribbean:

General Discussion

Erik Maitz Boman

7.1 Introduction

The heavy exploitation of queen conch throughout large parts of its natural range (Stoner 1997), as well as degradation of juvenile and adult habitats, has in many areas led to a reduction in population densities to unsustainable levels (Berg et al. 1992a, Berg et al. 1992b, Berg & Glazer 1995, Posada et al. 1997, Mateo et al. 1998, Delgado 1999, Chalifour 2010, Stoner et al. 2018). The decrease in landings of queen conch since its peak in the 1990s (Figure 1.3, **chapter 1**) has been attributed to overfishing, e.g. in Florida (Berg & Glazer 1995), and Bermuda (Berg et al. 1992a). However, temporary closure of some fisheries, such as in the Dominican Republic, Haiti, and Honduras (CITES 2003), as well as stronger management and conservation efforts (e.g. Pedro Bank, Jamaica), aiming to promote a more sustainable and productive fishery, have also contributed to the decrease in landings (Prada et al. 2017). Several biological characteristics (e.g. density-dependent mating and survival, slow-moving, a preference for shallow depths, and aggregating behavior during the reproductive season) make the species particularly vulnerable to overfishing (Appeldoorn et al. 2011). Consequently, many conch populations have experienced reduced densities to a point where mating success can be negatively affected (Stoner & Schwarte 1994, Berg et al. 1992a, Berg & Glazer 1995, Acosta 2006, Valle-Esquivel 2006, Stoner et al. 2018). Anthropogenic land-based activities in coastal areas has, in many places, had a negative effect on the habitat quality for conch (Sanders 1984, Posada et al. 1997, Glazer et al. 2008). In addition, the introduction and subsequent spread of a new invasive seagrass species (*H. stipulacea*) in the Caribbean in the early 2000s, has already caused significant alterations to the structure of native seagrass habitats, as well as in the associated trophic interactions (Scheibling et al. 2018). However, the impacts of this invasive seagrass on life-history parameters such as growth and survival remain unknown. Recovery of overfished populations has been slow (Theile 2001), although many Caribbean nations have implemented policies with regard to queen conch protection and exploitation. These measures however, are still mostly not harmonized among nations and often based on outdated and limited biological information (Stoner et al. 2012a) Queen conch fisheries management in the Caribbean is often complicated by internationally shared stocks and difficulties in data acquisition (Appeldoorn et al. 2011), which are partly caused by the logistically demanding and relatively expensive surveying of conch (Queen Conch Expert Workshop Group Report 2012, MRAG 2013). As the conventional survey methods using scuba

are limited to ca. 25 m depth due to safety limitations, these are not suitable for collecting data across the entire depth range of conch, which extends to 60 m.

The main objectives of this study were therefore, to address knowledge gaps in the biology and ecology of queen conch so that the distribution and dynamics of this species in relation to its environment are better understood. This will further expand our knowledge on marine gastropod biology in general, as well as on the effects of direct and indirect human-induced pressures on queen conch in the Caribbean. In addition, this study aimed to improve some methodological shortcomings (e.g. depth restriction, cost, safety of divers) in the surveying and assessment of queen conch. Ultimately, these findings could be instrumental in the management and conservation of the species. Thus, the following subjects were investigated:

1. Size-at-maturity and reproductive season of queen conch (**chapter 2**)
2. Development of a new survey tool to address methodological shortcomings in the surveying of queen conch (**chapter 3**)
3. Improve the prediction of abundance and distribution patterns of adult queen conch throughout the species' entire depth range (**chapter 4**)
4. The potential effects of the invasive seagrass (*H. stipulacea*) on juvenile queen conch (**chapter 5**)
5. Spatial genetic structure of queen conch in the Wider Caribbean Region (**chapter 6**)

In this chapter, the findings of these investigations are summarized and their limitations, biological and management implications are discussed. Suggestions for further research are offered in the context of current and future challenges for queen conch populations (e.g. climate change, habitat degradation, invasive seagrass).

7.2 Reproductive characteristics

In this study, I demonstrate a clear positive relationship between the thickness of the shell lip and the onset of maturity in queen conch, and that conch maturity occurs following the development of the lip (**chapter 2**). This is consistent with previous studies, which show that shell length growth stops before sexual maturation and is therefore not a reliable indicator of

maturity (Stoner & Sandt 1992, Stoner et al. 2012a). Queen conch were found to mature at a lip thickness of between 7 and 14 mm for females and between 4 and 11.5 mm for males. The use of a logistical model (Roa et al. 1999) to relate lip thickness with maturity showed that a previously widely-used accumulation model (Avila-Poveda and Baqueiro-Cardenas 2006, Bissada 2011, Stoner et al. 2012a) overestimated size-at-maturity for conch in the Caribbean (**chapter 2**). The present study results in better estimates of the size at which 50% of the population is mature (LT_{50}) and shows that conch mature at a smaller size than previously thought. The relationship between lip thickness and maturity varies in the Wider Caribbean Region, but not in relation to any temperature variable and did not follow the hypothesized temperature-size rule (Atkinson 1994), i.e. higher temperatures causing maturation at a smaller size. This may have been caused by insufficient differences in mean annual temperature differences and variation between the tropical Caribbean region (south of 23°N) and the sub-tropical Caribbean region (north of 25°N) (northern Bahamas).

In almost all locations females had a significantly larger lip thickness at 50% maturity (LT_{50}), which was consistent with the study of Foley and Takahashi (2017), who also found a different relationship between LT_{50} for female and male queen conch in Belize, using the same logistic model as in this study. In contrast to size-at-maturity, length of the reproductive season did show a relationship with temperature: locations with a relatively high variation in water temperature had a significantly shorter reproductive season compared to locations with low variation in water temperature (**chapter 2**). However, the length of the reproductive season defined in this and previous studies should not be regarded as a fixed parameter. Fishing can lower densities of adult conch below the minimum threshold for successful reproduction (60 mature conch/ha: Stoner & Ray- Culp 2000), and mask the true extent of the reproductive season, which would have occurred if densities of mature conch had remained high. Furthermore, due to variations in annual sea water temperatures, the length of the reproductive season can shift somewhat between years (Avila-Poveda and Baqueiro-Cárdenas 2009). Size-at-maturity estimates should be used with caution and are only truly applicable to the locations from which they were determined, which is apparent from the found differences between studied locations (Figure 3.1, **chapter 2**). Therefore, it would be useful to increase our understanding of spatial variability and the cause of variability of size-at-maturity throughout the region.

7.3 Abundance and distribution of adult queen conch

Distribution patterns of queen conch are complex, and studies have linked spatial distribution patterns of conch to habitat, depth, and other biotic and abiotic factors (Kough et al. 2018, Vallès & Oxenford 2012). Many studies have, however, excluded deeper parts (>25 m) of the survey area (Queen Conch Expert Workshop Group Report 2012, MRAG 2013). This was due to the difficulty of surveying areas with increased depth using conventional survey methods (scuba, snorkeling) (Queen Conch Expert Workshop Group Report 2012, MRAG 2013), and possibly also due to the notion of conch being more common at shallower depths (Ehrhardt & Valle-Esquivel 2008).

Thus, little priority was given to deeper sites, although deep-water conch populations are found throughout the Caribbean (Berg Jr 1975, Berg & Olsen 1989, Stoner & Sandt 1991, Appeldoorn 1997, Reynal et al. 2009, García-Sais et al. 2012). The assumed presence of deep-water populations and their role in replenishing shallow, more heavily fished populations has been used to develop harvest strategies, which were therefore based on potentially flawed stock assessments (MRAG 2013). Before examining how the spatial distribution of adult conch varies in response to a number of abiotic and biotic factors, a new survey method was developed, which was capable of surveying the entire depth range of conch (0-60 m), including deep-water adult populations. This was necessary due to the relatively deep (>25 m: all survey sites), and offshore site (Saba Bank) of the survey areas, making conventional survey methods impractical, costly and hazardous from a dive-safety perspective (Queen Conch Expert Workshop Group Report 2012, MRAG 2013). The rapid technical progress of video systems made it possible to develop new sampling tools to study benthic organisms, beyond depths safe for diving (Stevens 2003, Sheehan et al. 2010). A light, affordable, and safe towed video method (TVM), able to determine densities of adult queen conch throughout its entire depth range was thus developed and tested in this study. The new TVM was calibrated and compared with conventional belt transects (**chapter 3**). With the TVM it was possible to identify adult queen conch densities and accurately estimate adult queen conch densities in both low and high complexity habitats, as well as to provide a detailed habitat description of each transect (**chapter 3**). This method was successfully used to estimate queen conch densities to a depth of 57 m (**chapter 4**). Previously, concerns were raised regarding the

suitability of video methods for discerning between live and dead individuals (CRFM 2013), which is important to produce reliable abundance estimates and is critical for queen conch management (Ehrhardt & Valle-Esquivel 2006). However, I show here that this concern is unfounded for adult conch and that with TVM live adult conch can be accurately distinguished from dead individuals. The main limitation of the method is that it is not capable of navigating over large sharp objects higher than ca. 1 m (e.g. reefs, large boulders). The towed video method can also be challenging to operate in very shallow habitats (< 3 m), as the video array needs to be suspended at 1-3 m above the bottom depending on wave height. Furthermore, sensitive areas such as coral reefs could sustain some damage from the TVM, because of the drag chain which allows the frame to hover over the bottom. Therefore, in high relief and sensitive habitats as well as in very shallow water, other survey methods can be more suitable (**chapter 3**). The TVM developed in this study was not yet calibrated for intermediate and juvenile conch. Further calibration for the smaller size classes is required to determine the effectiveness of the TVM in estimating densities of these sizes of conch in different habitat complexities.

Based on the result of **chapter 3**, both a conventional survey method (i.e. belt transects using scuba) and the TVM were used to examine how the abundance and distribution pattern of adult queen conch varied in response to a number of biotic and abiotic factors (**chapter 4**). In addition, these data were used for studying spatial dependency in conch distribution patterns, which were modeled using Bayesian hierarchical spatial models (Integrated Laplace approximation: INLA) (Rue et al. 2009, Lindgren et al. 2011, Lindgren & Rue 2015). The spatially dependent models showed that adult conch occur in patchy distributions, with areas of high and low abundance caused by spatial dependency which in itself was not related to habitat, geomorphological area, season, or any other covariate included in the model. The present study suggests that spatial dependency is more likely to originate from endogenous processes, such as aggregating behavior, rather than exogenous processes (spatially structured environmental controls) (Planque et al. 2011). Stoner & Ray-Culp (2000) demonstrated an Allee effect in conch where reproductive activity declined at low densities of conch. Aggregation behavior during the reproductive season, which in many areas extends for more than half of the year, coupled with limited home range and mobility (Glazer et al. 2003, Delgado & Glazer 2007, Doer & Hill 2013, Stieglitz & Dujon 2017), likely maintains the patchy

distribution pattern, even outside the reproductive season. Consequently, conch are likely not able to utilize all suitable habitats (**chapter 4**), which would partly explain adult conch's rather weak and apparently inconsistent associations with environmental variables. However, this study did demonstrate that certain relationships between conch occurrence and environmental variables (i.e. depth, distance to open ocean, substrate cover, macroalgae cover) are present, although they are seldom linear and mostly show large variation, which makes predicting conch distribution patterns challenging.

In the survey sites (St Eustatius, Saba Bank, and Anguilla) adult conch were found at all surveyed depths (4-57 m), which is consistent with the reported depth range of conch (0-60 m) (Randall 1964). Abundance patterns in relation to depth differed between areas, but the highest densities (>100 / ha) of adult conch were always found in deeper waters (17 – 45 m), and at Saba Bank high densities of 300 adult conch per ha were found at a depth of 40 m, both within and outside the peak reproductive season (**chapter 2**). Similar high densities of adult conch have been observed in deep water (ca. 30 m) habitats during the peak reproductive season in Puerto Rico (Garcia-Sais et al. 2012). The skew toward a deeper distribution of adult conch with only high densities at greater depths at the three survey sites was likely influenced by different factors at the different areas. Saba Bank has a moratorium on conch fishery since 1994, thus little influence is expected from fisheries, but it also has a minimum depth of 15 m, which automatically skews the depth distribution of conch at this location. In both Anguilla and St Eustatius an artisanal fishery is active, potentially depleting conch at shallower depths, especially in Anguilla, where the fishing pressure is higher than in St Eustatius (6% of total adult population in Anguilla: Kuramae Izioka 2016, and 3 % in St Eustatius: Meijer zu Schlochtern 2014). Although the fishing pressure at Anguilla was lower than the 8 % recommended by the Queen Conch Expert Workshop Group Report (2012), the higher fishing pressure was reflected in the lowest overall mean density of adult conch (26 / ha) at Anguilla, compared to St Eustatius (62 / ha), and Saba Bank (126 / ha). Besides fishing, in St Eustatius habitat distribution, with much of the reef habitats located in the shallow areas around the island, and the geomorphology of the island which offers little protection from the elements (i.e. wind, waves) in the shallower areas (< 15 m), likely contributed to the depth distribution. As at all three survey sites, high adult densities with a high probability of mating success (>90 % probability: Stoner & Ray-Culp 2000, Stoner et al. 2012d) were only found in deeper waters,

this stresses the importance of deep-water adult conch for the overall reproductive output and capacity.

The Bayesian models in this study showed that macroalgae cover has an important effect on adult conch abundance and provides a first indication that there is an optimal range of macroalgae cover (ca. 20 – 60 %) for adult conch (**chapter 4**). I hypothesize that even macroalgae cover of nondietary significance (which was the majority of identified algae in the transect for all locations) can, to a certain extent, be beneficial for adult conch by stabilizing sediment, creating favorable conditions, and increasing the available growth area for other sources of nutrition (i.e. benthic diatoms, epiphytes). However, above a certain cover (ca. 60-70%), macroalgae may instead inhibit an important source of nutrition (i.e. benthic diatoms) through light competition (Hill 1996, Yang & Flower 2012), which cannot be fully compensated by the available epiphytic food sources growing on the macroalgae. Another important relationship was found between adult conch abundance and distance to the open ocean on Saba Bank. Here, abundance of conch was higher towards the edge of the bank, which could be caused by a preference for habitats with a higher water flow compared to the center of the bank. Although this study did not measure water flow directly, a positive correlation with higher water flow towards the edge of the bank and closer to the open ocean is present (Hamner & Hauri 1981). A previous study (Kough et al. 2018) similarly found a positive relationship between channels with high tidal current flow and adult conch abundance. Although the mechanism for the positive association between adult conch and increased water flow has not been determined, the presence of adult conch in areas with higher water flow could lead to increased larval dispersal (Sponaugle et al. 2002). Similar strategies to increase larval dispersal are reported in other marine fish and invertebrates with a planktonic phase, in which spawning adults show a preference for areas with higher and lower water flow, which can promote dispersal or retention of larvae respectively (Sponaugle et al. 2002). This pattern of increased abundance towards the edges of the Bank was partly offset by the presence of unsuitable habitat at the very edge of the Bank, which was covered by high-relief reef, causing a lack of conch in this area.

Similar associations between distance to the open ocean and conch abundance were not found around the two island locations in the study (Anguilla, St Eustatius). However, this is

also not to be expected as water flow patterns around islands are complex and not correlated with distance to the open ocean as they are on banks (Hamner & Hauri 1981). Furthermore, due to the limited space around many small islands in the Caribbean, relocation to optimal habitats with increased water flow is often not possible for adult conch (**chapter 4**) (FAO 1993).

7.4 Effect of the invasive seagrass *Halophila stipulacea*

Seagrass habitats have long been known to be important for conch, in particular for juveniles, that, in many parts of the Caribbean, are primarily associated with native seagrass beds, which provide both nutrition and protection from predators (Ray & Stoner 1995, Stoner 2003, Stoner & Davis 2010). Alterations to the composition of seagrass beds, such as through the introduction of invasive species (e.g. *H. stipulacea*), can cause changes in associated biota and change the level of shelter and the quality and quantity of food sources (Willette & Ambrose 2012). This study shows that the diet of juvenile conch is comparable in native and invasive seagrass beds, but that growth rates were lower in invasive, compared to native seagrass beds. Stable isotope analysis showed that organic material in the sediment (benthic diatoms and POM) was the most important source of nutrition for all examined habitats, despite differences between habitat factors such as seagrass composition, depth, distance to shore, and anthropogenic nutrient input (**chapter 5**). The importance of organic material in the sediment for juvenile queen conch was suggested by Stoner & Waite (1991), who found large amounts of sediment in the stomach of juvenile conch. These authors further suggested a high nutritional importance of *T. testudinum* in the diet of juvenile conch. However, the results of our study suggest that the nutritional importance of native seagrass detritus for juvenile conch may be less prominent than previously thought, and that detritus from the invasive seagrass *H. stipulacea* is likely to be a very limited source of nutrition. Therefore, it is doubtful that detritus of the invasive seagrass can act as an important food source in areas where a shift from native to invasive seagrass has occurred. The growth experiment also indicated that juvenile growth is reduced in invasive compared to native seagrass beds, at least under conditions where other sources of nutrition were not available (**chapter 5**). I hypothesized that this is due to the growth pattern of the invasive seagrass, which is much denser compared

to native seagrass (Tussenbroek et al. 2016, Linton & Fisher 2004, Becking et al. 2014) and thus could limit access to the sediment, as well as creating unfavorable conditions for benthic diatoms due to light competition (Hill 1996, Yang & Flower 2012). Consequently, the invasion of *H. stipulacea* could lead to fewer food sources of improper nutritional quality, negatively affecting juvenile growth. Unfortunately, this hypothesis could not be completely tested during the course of this study (**chapter 5**) due to the sudden obliteration of the study site and consequent end of the experiment with the passing of Hurricane Irma (September 2017).

It is to be expected that the invasion of *H. stipulacea* will not be limited to its current distribution range, which includes the Eastern Caribbean and northern South America (Willette et al. 2014, Vera et al. 2014). How the spread of *H. stipulacea* will influence the population dynamics of conch in the Wider Caribbean Region is uncertain at this stage, but this study suggests that negative effects on growth, and consequent survival of juvenile conch are likely, which would affect carrying capacity within these areas. Survival of juvenile conch is positively correlated with density (safety in numbers), whereas growth is negatively correlated with density (intra-specific competition) (Weil & Laughlin 1984, Stoner & Ray 1993). If carrying capacity is reduced in invasive compared to native seagrass beds, as the results of this study indicate, fewer conch per unit area will be able to be sustained compared to medium-density native seagrass beds.

7.5 Conch connectivity

Population dynamics of a species also include immigration and emigration. For queen conch this mainly pertains to the dispersal of larvae as the relative mobility of settled conch is limited (Glazer et al. 2003, Delgado & Glazer 2007, Doer & Hill 2013). The present study shows that queen conch does not form a single panmictic population in the greater Caribbean, but that there are significant levels of genetic differentiation between and within Caribbean countries, supporting previous genetic studies which hypothesized that clinal patterns in haplotype frequencies were caused by isolation through oceanic distance and Caribbean ocean currents (Mitton et al. 1989, Perez- Enriquez et al. 2011) (**chapter 6**). This isolation occurs despite a reported potential dispersal range of hundreds of kilometers (Appeldoorn 1994, Stoner &

Davis 1997), although other empirical observations have indicated that the dispersal range of conch may be more limited (Posada & Appeldoorn 1994, de Jesús-Navarrete & Aldana-Aranda 2000, Delgado et al. 2008). Similarly, genetic differences in other gastropod and bivalve species have been explained by ocean currents and isolation by oceanic distance (Zhan et al. 2009, White et al. 2010). Overall, the findings of this study provide evidence of a significant spatial genetic structure that is explained by isolation by oceanic distance. This means that immigration and emigration of conch are limited by oceanic distance, and that queen conch populations cannot rely solely on external sources of larvae to support low-density populations that are unable to produce sufficient numbers of their own recruits. This may impede the natural recovery of overfished conch populations and could be a key factor towards explaining why the Florida Keys fishery has yet to rebound after 30 years of closure (Delgado et al. 2008). Previous research has suggested that hydrodynamic processes act as retentive mechanisms for locally produced queen conch larvae in the Florida Keys, and that few larvae are coming from upstream populations outside of the Florida Keys (Delgado et al. 2008). Further evidence to support the hypothesis of isolation by oceanic distance comes from population declines in the Exuma Cays Marine Protected Area in The Bahamas, despite decades of protection and high levels of compliance within the protected area (Stoner et al. 2012a). Although the spatial genetic structure of queen conch was characterized across the greater Caribbean seascape, using nine microsatellites and sampling 643 individuals from 19 locations, it was not possible to delineate precise stock boundaries in this study. Moreover, the findings of this study, from population-level analyses, should be interpreted with caution (Christie et al. 2010), as these rely upon theoretical assumptions (e.g., Hardy–Weinberg equilibrium and drift-mutation equilibrium), which limit the statistical power to detect some ecological relevant patterns of connectivity when faced with moderate to high levels of gene flow (Hellberg 2009).

7.6 Progress in conch knowledge, future research directions and implications for conservation and management

This study has provided new insights into queen conch biology and population dynamics and also led to the development of a new method to survey queen conch populations throughout the species' entire depth range. The towed video method (TVM) is capable of estimating adult

queen conch densities, also at deeper sites without increased cost and risk to divers (**chapter 3**). When deeper sites (>20 m) are part of the survey area, or if stock estimates are based on the assumed presence of a deep-water spawning stock that replenishes shallow-water stocks (MRAG 2013), such sites should always be included in the stock estimation, which is now feasible through this new methodology. The importance of including the deep-water stock was illustrated by our surveys at three locations in the eastern Caribbean (Anguilla, St Eustatius, Saba Bank: **chapter 4**). High densities of adult conch (>100 / ha) were only present in deeper water (ca. 17 – 45 m) at all three locations. If surveys at these locations would have been conducted using only conventional survey methods (i.e. scuba or snorkelling), restricted to depths <25 m, they would have failed to estimate distribution patterns of conch in the deeper areas (< 25 m). The use of our new TVM method therefore enables more reliable population estimates. At present, a combination of both TVM and conventional methods such as belt transects is recommended for surveying of queen conch so that both shallow and deep-water populations can be surveyed effectively. However, future technical progress of video systems, as well as post-video analysis software, will likely make TVM even more appealing regarding cost-effectiveness and increased accuracy, which could decrease the importance of conventional methods, thus saving time and costs.

In addition to overall better abundance estimates, this study provided evidence of a general patchy distribution of conch with spatial dependency, which was mostly independent of habitat variables. This implies that conch distribution is not only influenced by habitat, but also by endogenous processes such as aggregating behavior. Future studies should take spatial dependency into account, since ignoring it may result in biased population estimates (Zuur et al. 2017), and may point to conclusions radically different from those suggested by spatially independent models (Carroll & Pearson 2000, Keitt et al. 2002).

This study also provides an important framework for more biologically sensible minimum-size regulations for the management of conch fisheries. At present, most Caribbean nations have minimum-size regulations which are not effective in ensuring that at least 50% of the queen conch population will reach maturity before being harvested (**chapter 2**). Most of these regulations are based on shell length, which was proven to be an unreliable indicator of maturity by this and previous studies. In the >30 nations where conch fishing is allowed, only

a few (i.e. Antigua and Barbuda, Cuba, Nicaragua, Puerto Rico, St Barthélemy, and US Virgin Islands) have specified minimum size regulations regarding lip thickness, ranging from 5 to 9.5 mm. Although size regulation based on lip thickness is far superior over shell length in terms of protecting mature individuals, most lip thickness regulations are close to the lower estimation range for maturity in conch found in this study. Moreover, the mathematical model used to estimate size-at-maturity (the accumulation model) was shown to overestimate size-at-maturity and could be inappropriate for this purpose. The alternative logistical model was proposed which can estimate size-at-maturity more accurately, and is the model generally used for this purpose.

In addition, this study showed that a minority of Caribbean nations have a closed fishing season which overlaps with the peak reproductive season of conch (**chapter 2**). Although closing of the fishing season is a commonly applied measure to protect spawning stocks, the current use of this measure is less than optimal. I found that the reproductive season of queen conch occurs simultaneously in the vast majority of locations in the Wider Caribbean Region between May and September, and that a Caribbean-wide closure within this period would maximize the protection of spawning conch in most locations.

The findings from the diet and growth experiment in native and invasive seagrass beds demonstrate that juvenile conch have similar feeding patterns in all habitats, despite differences in environmental conditions, with organic material in sediment (benthic diatoms and POM) being the most important source of nutrition. Growth, however, was lower in invasive compared to native seagrass, likely due to the dense growth pattern of invasive seagrass which limits access to the most important nutrition source (benthic diatoms and POM) (**chapter 5**). The implications for decreased growth of juveniles in invasive seagrass beds would be decreased population growth and a lower resilience to fishing pressure. Due to the rapid spread of the invasive seagrass *H. stipulacea*, and its continued advancement west and north in the Caribbean, many more areas will soon experience a complete or partial shift in seagrass composition towards the invasive seagrass (Willette et al. 2014). Slower population growth in areas where *H. stipulacea* has invaded will require adjustments of harvest strategies and quotas, matching the growth capacity of the population to avoid stock depletion. The results of the experiment on individual growth of juvenile conch were not completely

conclusive yet, since the experiment had a shorter than desired duration. It is uncertain if the presence of other sources of nutrition, which were not present in this study (e.g. cyanobacteria, or macro-algae such as *Laurencia* spp. or *Batophora oerstedii*), could compensate for the limited access to sediment. It is therefore crucial to further investigate differences in growth and diet between native and invasive habitats. In addition, changes in other life-history traits, such as survival should be investigated under different seagrass species compositions and densities, as well as in the presence of alternative food sources. This would provide better knowledge of the expected effects of invasive seagrass as it becomes more widespread and would help adjust management of already affected stocks. Managers in currently unaffected areas would thus be given an opportunity to adjust their management strategies before *H. stipulacea* reaches those areas.

The findings of spatial genetic structure studies demonstrate that queen conch consists of several regionally isolated subpopulations throughout the greater Caribbean and is constrained by oceanic distance (**chapter 6**). This suggests that management of queen conch harvest quotas should be tailored towards a more localized stock structure, particularly in regions with high levels of genetic divergence such as the Caribbean Netherlands, Mexico, and Florida. Many Caribbean nations, especially in the Eastern Caribbean, are small islands often in close proximity to each other. Conch stock boundaries are thus unlikely to follow geopolitical boundaries, and coordinated stock management could be beneficial in these locations. Coordinated international management will also be required to maintain population connectivity over spatial scales that span international boundaries in the remote offshore network of banks in the Nicaraguan rise, which includes the territorial seas of Honduras, Nicaragua, Colombia, and Jamaica. Further studies in these regions using next-generation high-resolution genomic techniques will be useful to delineate stock boundaries and subpopulations more accurately, as well as provide information regarding necessary management cooperation.

7.7 Future challenges and prospects for queen conch

It is evident, based on the results of this and previous studies (Glazer & Quintero 1998, Kough et al. 2018, Stoner et al. 2018) that queen conch have in the past, are currently, and will in the future, face similar and new challenges (e.g. fishing, habitat degradation, invasive species, climate change). For instance, fishing has caused an increase in mortality and has consequently decreased queen conch population densities in some areas to unsustainable levels, at which mating success is negatively affected (Delgado 1999, Berg & Glazer 1995, Mateo et al. 1998, Stoner et al. 2018), leading to even lower population levels (Allee effect: Stoner & Ray-Culp 2000). Habitat degradation, caused by human activity (e.g. sedimentation, eutrophication, pollution), has also led to both a decline in reproductive output (Glazer & Quintero 1998) and an increase in mortality (Sanders 1984). Effects of these challenges (e.g. high fishing pressure) on the population dynamics are in many cases predictable. However, future challenges and changes in abiotic and biotic factors due to climate change and the introduction of invasive species are more uncertain and often largely unknown. Possible effects on conch life-history parameters through future changes to abiotic factors have been discussed by Appeldoorn & Baker (2013), mainly pertaining to increasing sea temperatures and acidification. Acidification may cause an increase in the cost of shell production and limit the depth of shell formation (Doney 2006), while a rise in sea temperature will likely cause changes to a range of conch life-history parameters. For example, the length of the reproductive season is to a large extent regulated by variation in yearly temperature (**chapter 2**), and during especially warm years conch can reproduce throughout the year (Randall 1964). Heat stress has also been observed in conch, with a decrease in reproductive behaviors at extreme temperatures ($> 30.5^{\circ}\text{C}$) (Appeldoorn et al. 2011). Locally, the duration and intensity of the reproductive season are likely to change. More frequent episodes of extreme temperatures can also be expected (Christidis et al. 2011, Duffy & Tebaldi 2012), and consequently, an increased frequency of temporary disruptions to the reproductive season due to heat stress is likely unless conch can adapt to the increase in seawater temperature. However, whether this will affect total reproductive output and survival of larvae is unclear. Size at maturity was not found to follow the hypothesized temperature-size rule in this study (**chapter 2**), possibly due to temperature differences not being large enough between the tropical parts and the sub-tropical areas of the Caribbean to noticeably affect size at maturity

in queen conch. However, it cannot be ruled out that size at maturity will be affected by long-term changes in seawater temperature. Increased temperatures can also be expected to increase conch growth rates (Appeldoorn & Baker 2013) as well as possibly accelerating larval development (Rodriguez Gill 1995) when metabolic rate increases. However, growth rates will only increase if individuals can consume more food at a higher temperature to compensate for the increase in metabolic rate (Jobling 1997). Increase in shell growth rate has been observed in mollusks when the increase in temperature was within the normal range for the species (*Pinctada margaritifera*) (Joubert et al. 2014), but there was a negative impact on shell growth when the temperature rose above the ecological limits of the species (Pouvreau & Prasil 2001). Thus, with rising sea temperatures it is likely that seasonal growth patterns will shift, with an increase in conch growth rate during the cooler months of the year when the increase in temperature is likely still within the normal range of the species, whilst during the warmest months, when conch currently experience their highest growth rates, growth could be hampered (Randall 1964, Weil & Laughlin 1984, Iversen et al. 1987, Wicklund et al. 1988, Stoner 1989a,b, Stoner & Sandt 1992, Appeldoorn et al. 2011). Furthermore, changes in the strength and direction of ocean currents due to climate change (Bryden et al. 2005, Caesar et al. 2018, Thornalley et al. 2018) may impact conch and other marine species with a planktonic larval phase (Hays 2017). This has been observed in eel species *Anguilla anguilla* (Baltazar-Soares et al. 2014) and *Anguilla japonica* (Chang et al. 2018), whereby changes in ocean circulation have affected larval migration success to their recruitment areas. The current spatial genetic structure of queen conch is probably shaped by hydrodynamic processes (e.g. currents, wave, wind) that can act as retentive mechanisms for larvae and cause isolation by oceanic distance (**chapter 6**). Thus, changes in hydrodynamic processes due to climate change could change immigration and emigration patterns and the spatial genetic structure of conch, as seen in other marine species (e.g. *A. japonica*) with planktonic larval phase (Baltazar-Soares et al. 2014, Chang et al. 2018). Higher intensification of tropical storms and increased precipitation as a result of warmer sea temperature (Webster et al. 2005, IPCC 2007, Lough 2007) can locally have severe effects, particularly on conch populations in shallow waters. Unfortunately, this was experienced in the current study, in which all conch and much of the macrophyte cover inside Simpson Bay (0-10 m, ca. 2 km²), St Maarten, were removed after the passing of category 5 Hurricane Irma on 6 September 2017 (**chapter 5**). Increased

precipitation is also likely to increase the amount of runoff into nearshore habitats, where conch nurseries are often located (Randall 1964, Weil & Laughlin 1984, Iversen et al. 1987, Stoner 2003), reducing habitat quality in affected areas.

Although changes in the above-mentioned factors are likely to severely alter the population dynamics of queen conch in the long term and thus warrant attention, there are also more short-term threats. One of the most acute phenomena is the upsurge of the invasive seagrass *H. stipulacea*, which is likely to affect the population dynamics of queen conch populations throughout the Wider Caribbean Region in the immediate future. Previously to this study (**chapter 5**), no research regarding the impact of *H. stipulacea* on conch had been conducted, possibly due to the fact that this seagrass had not yet been found in any of the primary countries with industrial or extensive artisanal conch fisheries (i.e. Jamaica, Colombia, Cuba, Honduras, Nicaragua, Belize, Turks and Caicos, and the Bahamas). Due to the specific habitat requirements of juvenile queen conch (Stoner et al. 1994) and their association with native seagrasses such as *Thalassia testudinum* (Stoner 2003), alterations to these important habitats could have been expected to invoke a greater interest from the conch community. However, the results of this study suggest that juvenile conch growth is lower in invasive compared to native seagrass beds (**chapter 5**), and greater attention to this possible threat from the conch community should be expected and demanded. Although much uncertainty still exists regarding the effects of *H. stipulacea* on the population dynamics on queen conch, if lower growth rates in invasive seagrass beds is a general pattern, it would have ramifications for both births and deaths of conch and the overall carrying capacity of conch populations in the Caribbean. Queen conch will in the future face new challenges, expected to change abiotic and biotic factors and in turn cause short and long-term changes to many of its life-history parameters (e.g. size-at-maturity, reproductive season, growth rate, spatial genetic structure). Consequently, such parameters should not be considered rigid, putting an unknown time limit to the relevance of the current biological knowledge of these parameters. It is also, uncertain to what degree queen conch and other species can adapt to environmental changes induced by climate change and invasive species (Hoffmann & Sgrò 2011, Ricciardi 2003, Ricciardi & Kipp 2008). Therefore, commitment to long-term research and updates in current biological knowledge, life-history parameters and population dynamics of queen conch throughout its

range will be required to adjust subsequent management and conservation strategies to ensure the long-term sustainability of the species.

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Summary

Summary

The marine gastropod queen conch (*Lobatus gigas*), found throughout the Wider Caribbean Region, supports one of the most important fisheries in the region. However, several biological characteristics (e.g. density-dependent mating and survival, slow-moving, a preference for shallow depths, and aggregating behaviour during the reproductive season) make the species particularly vulnerable to overfishing. The heavy exploitation of queen conch throughout large parts of its natural range, as well as degradation of juvenile and adult habitats, also has in many areas led to a reduction in population densities to unsustainable levels to a point where mating success can be negatively affected. In addition, a new invasive seagrass species (*H. stipulacea*) has already caused significant alterations to the structure of native seagrass habitats which are in many parts of the Caribbean important to juvenile conch, providing both nutrition and protection from predators. However, the impacts of this invasive seagrass on life-history parameters such as growth and survival remain unknown.

Although many Caribbean nations have implemented policies with regard to queen conch protection and exploitation, recovery of overfished populations has been slow. These measures, however, are still mostly not harmonized among nations and often based on outdated and limited biological information. Management of queen conch is also often complicated by difficulties in data acquisition, partly caused by the logistically demanding and relatively expensive surveying of conch. Conventional survey methods using scuba are also limited to ca. 25 m depth due to safety limitations, making them unsuitable for collecting data across the entire depth range of conch, which extends to 60 m.

The main objectives of this study were to address knowledge gaps of the biology and ecology of the queen conch so that the distribution and dynamics of this species in relation to its environment are better understood. Such information will further improve our knowledge of marine gastropod biology in general, as well as our understanding of the effects of direct and indirect human-induced pressures on queen conch in the Caribbean. In addition, this study aimed to tackle some methodological shortcomings in the surveying and assessment of queen conch. Ultimately, these findings could be instrumental in the management and conservation of the species.

Reproductive characteristics are important biological reference points for the management of species. To improve the knowledge of queen conch reproductive biology (i.e. size-at-maturity and reproductive season), evaluation and comparison of the relationship between shell lip thickness and maturity in queen conch throughout the Wider Caribbean Region, using histological analysis of queen conch gonads was carried out. Furthermore, the influence of seawater temperature on the length of the reproductive season was investigated (**chapter 2**). We demonstrate a clear positive relationship between the thickness of the shell lip and the onset of maturity in queen conch, and that maturity occurs following the development of the lip. Lip thickness at 50% maturity (LT_{50}) of both females and males varied between different locations in the Caribbean, although it did not correspond with variation in water temperature. In most cases, females had a larger LT_{50} than males indicating sexual dimorphism. Locations with a relatively high variation in water temperature had a significantly shorter reproductive season. The implementation of adequate minimum size regulation based on lip thickness (ca. 15 mm) and a Caribbean wide seasonal closure (May–September) using the most recent biological information from this study, taking into consideration the local differences in LT_{50} and reproductive season, will assist in developing a long-term sustainable queen conch fishery in the Caribbean.

To address the methodological shortcomings in the surveying of queen conch, a novel towed video method (TVM) was developed and compared with a conventional survey method (i.e. belt transect [BT] using scuba divers) in a series of calibration transects in two different habitats (i.e. high complexity (HC) and low complexity (LC)) (**chapter 3**). In both habitats, adult live queen conch had similar counts with both methods. Adult dead conch were not mistaken for live conch and the results validate the use of TVM as a reliable sampling tool to estimate densities of live adult conch in both HC and LC habitats throughout the species' depth range.

In **chapter 4**, the spatial distribution of adult queen conch and how it varies in response to a number of known abiotic and biotic variables between sites which vary in environmental conditions was examined. By combining TVM with conventional belt-transects, a more comprehensive survey of conch abundance was performed at three sites in the Eastern Caribbean (Anguilla, St Eustatius, Saba Bank). Adult conch appeared in patchy distributions, mostly caused by spatial dependency, which was likely related to aggregating behaviour

during spawning events. Environmental variables, such as algae cover, distance to the open ocean, and depth showed important non-linear effects on conch abundance, although these differed among sites. The proportion of reef and sand cover had important negative effects on conch abundance at all sites. High densities (>100 /ha) of adult conch were found only at depths >17 m at all three sites. The lack of strong generic location over-crossing relationships between abiotic and biotic factors and adult conch abundance and distribution is likely partly due to this spatial dependency, as well as different location-specific factors that affect different stages of the conch's life-history. Furthermore, the results indicate that intermediate and deep areas (ca. 17 – 45 m) contain most of the reproductive output of conch in the survey sites and are therefore highly important for reproductive capacity. Thus, surveying areas at depths beyond the practical limitation of divers (<25 m) are of great importance to obtain more reliable population estimates.

To provide a first insight into the possible impact of an invasive seagrass species (*H. stipulacea*) on queen conch, the diet and growth of juvenile conch in both native, mixed, and invasive seagrass beds was examined using stable isotope analysis and an *in situ* growth enclosure experiment (**chapter 5**). Organic material in the sediment (i.e. benthic diatoms and particulate organic matter [POM]) was found to be the most important source of carbon and nitrogen for juvenile queen conch in all three habitats investigated, and there was a significantly higher probability of positive growth in the native seagrass compared to the invasive seagrass. Due to the importance of the organic material in the sediment as a source of nutrition for juvenile conch, limited access to the sediment in the invasive seagrass can potentially cause inadequate nutritional conditions to sustain high growth rates. Thus, it is likely that there is a negative effect on juvenile queen conch growth currently inhabiting invasive seagrass beds, compared to native seagrass beds, when other potential sources of nutrition are not available. Although much uncertainty still exists regarding the effects of *H. stipulacea* on the population dynamics on queen conch, if lower growth rates in invasive seagrass beds is a general pattern, it would have ramifications for both births and deaths of conch and the overall carrying capacity of conch populations in the Caribbean.

A better understanding of the spatial genetic structure (SGS) and the factors driving contemporary patterns of gene flow and genetic diversity of queen conch are fundamental for

developing conservation and management plans for marine fisheries. A detailed study of SGS and genetic diversity was therefore performed using population genetic and multivariate analyses (**chapter 6**). Our study found that queen conch does not form a single panmictic population in the greater Caribbean. Significant levels of genetic differentiation were identified between Caribbean countries, within Caribbean countries, and among sites. Gene flow over the spatial scale of the entire Caribbean basin is constrained by oceanic distance, which may impede the natural recovery of overfished queen conch populations. Our results suggest a careful blend of local and international management will be required to ensure long-term sustainability for the species.

This study has provided new insights into queen conch biology and population dynamics as well as methodological shortcomings so that the distribution and dynamics of this species in relation to its environment are better understood. Ultimately, the findings from this study can contribute to improving the management and conservation of the species. However, the species will in the future face new challenges, due to expected changes in abiotic and biotic factors, such as temperature, ocean currents, and seagrass species composition. As body temperature and thus their physiological functions (e.g. growth) are directly dependent on environmental condition in this ectotherm species, it is particularly vulnerable to climate change (Dillon et al. 2010). Consequently, life-history parameters (e.g. size-at-maturity, reproductive season, growth rate, spatial genetic structure) of queen conch should not be considered rigid as these can be expected to change in the short and long-term, putting an unknown time limit to the relevance of the current biological knowledge of these parameters. However, there is still much uncertainty regarding what degree queen conch and other species can adapt to environmental changes induced by climate change and invasive species. Therefore, commitment to long-term research and updates in current biological knowledge, life-history parameters and population dynamics of queen conch throughout its range will be required to adjust subsequent management and conservation strategies to ensure the long-term sustainability of the species.

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Project title Biology, monitoring, and management of a tropical marine gastropod: the Queen conch (*Lobatus gigas*) in the Caribbean



Group Aquaculture and Fisheries (AFI)

A. The Basic Package
3 ECTS

WIAS Introduction Day
Course on essential skills
Research ethics

B. Disciplinary Competences
18.6 ECTS

Writing own research proposal
Fisheries Ecology (WAIS Specialization Marine Biology)
Bayesian Statistics
Introduction to zero-inflated models with R
Modern methods in data analyses

C. Professional Competences
1.5 ECTS

The essential of scientific writing and publishing
Scientific publishing

D. Presentation Skills
3 ECTS

A survey of deep-water Queen conch (*Lobatus gigas*) populations using a towed video array, Barbados (GCFI), 7th November 2014, oral presentation

Queen conch reproduction, Saba (Sea and Learn) 2015, oral presentation

Size at maturity and reproductive season of Queen conch in the greater Caribbean region, St Eustatius (CNSI), Nov 2016, oral presentation

E. Teaching competences
6 ECTS

Supervising BSc thesis (x 2)
Supervising MSc thesis
Teaching field course (Sustainable island management, van Hall Larenstein)
Teaching field course (Sustainable island management, van Hall Larenstein)

Education and Training Total
32.1 ECTS

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